(19)

(11) **EP 1 780 217 B1**

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent: **31.03.2010 Bulletin 2010/13**

(51) Int Cl.: *C07K 14/47* (2006.01) *C12N 15/12* (2006.01) *C12Q 1/68* (2006.01)

(21) Application number: **06026286.2**

(22) Date of filing: **31.10.2000**

(54) **Molecular structure of RHD negative locus**

Molekularstruktur des RHD-negativ Genortes

Structure moleculaire de lieu de RDH négatif

(84) Designated Contracting States:
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE**

(30) Priority: **02.11.1999 EP 99121686**
**31.05.2000 EP 00111696**

(43) Date of publication of application:
**02.05.2007 Bulletin 2007/18**

(62) Document number(s) of the earlier application(s) in accordance with Art. 76 EPC:
**00972884.1 / 1 226 169**

(73) Proprietor: **DRK-Blutspendedienst Baden-Württemberg-Hessen gemeinnützige GmbH**
**89081 Ulm (DE)**

(72) Inventors:
- **Flegel, Willy A.**
  **64807 Dieburg (DE)**
- **Wagner, Franz F.**
  **31832 Springe (DE)**

(74) Representative: **Vossius & Partner**
**Siebertstraße 4**
**81675 München (DE)**

(56) References cited:
**WO-A-99/37763 WO-A-99/47555**
**US-A- 5 972 602**

- **CARRITT, B. ET AL.: "Evolution of the Rh (rhesus) blood group genes: a 50 year old predication (partially) fulfilled" HUMAN MOLECULAR GENETICS., vol. 6, no. 6, 1997, pages 843-850, XP000998694 OXFORD UNIVERSITY PRESS, SURREY., GB ISSN: 0964-6906**
- **CARRITT, B. ET AL.: "Rh null phenotypes are not due to a gross deletion and can occur on different Rh genetic backgrounds" ANNALS OF HUMAN GENETICS, vol. 57, no. 4, 1993, pages 273-279, XP000926510**
- **OKUDA H ET AL: "SEQUENCE ANALYSIS OF THE SPACER REGION BETWEEN THE RHD AND RHCE GENES" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS., vol. 263, 24 September 1999 (1999-09-24), pages 378-383, XP000998148 ACADEMIC PRESS INC. ORLANDO, FL., US ISSN: 0006-291X**
- **HU, G.: "Human small membrane protein 1 (SMP1) mRNA" EMBL SEQUENCE DATABASE, 4 March 1999 (1999-03-04), XP002170737 HEIDELBERG DE**
- **LE VAN KIM CAROLINE ET AL: "Molecular cloning and primary structure of the human blood group RhD polypeptide." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 89, no. 22, 1992, pages 10925-10929, XP002134526 1992 ISSN: 0027-8424**
- **CHERIF-ZAHAR B ET AL: "Organization of the gene ( RHCE) encoding the human blood group RhCcEe antigens and characterization of the promoter region." GENOMICS, vol. 19, no. 1, 1994, pages 68-74, XP000926511 ISSN: 0888-7543**
- **WAGNER, F.F. ET AL.: "RHD gene deletion occured in the Rhesus box" BLOOD, vol. 29, no. 12, 15 June 2000 (2000-06-15), pages 3662-3668, XP000926502 WASHINGTON US**

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).



**(Cont. next page)**

- BLUNT, T. ET AL.: "Serotype switching in a partially deleted RHD gene" VOX SANGUINIS, vol. 67, no. 4, 1994, pages 397-401, XP000926512
- AVENT ND ET AL: "Evidence of genetic diversity underlying Rh D-, weak D (Du), and partial D phenotypes as determined by multiplex polymerase chain reaction analysis of the RHD gene" BLOOD, W.B. SAUNDERS, PHILADELPHIA, VA, US, vol. 89, no. 7, 1 April 1997 (1997-04-01), pages 2568-2577, XP002108680 ISSN: 0006-4971
- DATABASE EM_HUM [Online] EMBL; 13 August 1998 (1998-08-13), COBLEY. V. ET AL.: "Human DNA sequence clone RP3-469D22 on chromosome 1p35.1-36.13, contains the 5'part of the gene for RHCE." XP002178290 retrieved from EBI accession no. HS469D22 Database accession no. AL031284
- WAGNER FF ET AL: "Molecular basis of weak D phenotypes" BLOOD, W.B. SAUNDERS, PHILADELPHIA, VA, US, vol. 93, no. 1, January 1999 (1999-01), pages 385-393, XP002108682 ISSN: 0006-4971
- OKUDA H. ET AL.: "The RHD gene is highly detectable in RhD-negative Japanese donors" JOURNAL OF CLINICAL INVESTIGATION, vol. 100, no. 2, July 1997 (1997-07), pages 373-379,

## Description

**[0001]** The present invention relates to a nucleic acid molecular structure representing the Rhesus genes locus comprising the *RHD* gene. Furthermore, the invention relates to a process for the specific detection of the common *RHD* negative haplotypes. The invention further relates to the detection of *RHD* positive haplotypes in D-negative individuals. Various mutations in the *RHD* gene have been identified that allow for the development of diagnostic tools. The invention also relates to oligonucleotides. Additionally, the invention relates to kits comprising or employing the above recited compounds of the invention.

**[0002]** The Rhesus D antigen (ISBT 004.001; RH1) is the most important blood group antigen determined by a protein. Anti-D remains the leading cause of hemolytic disease of the newborn (Filbey, Acta Obstet Gynecol Scand, 74:687,1995; Bowman, J, Semin Perinatol 21:39, 1997). Depending on the population, 3% to 25% of whites lack the antigen D (Mourant, The distribution of the human blood groups and other polymorphisms, London, Oxford University Press, 1976). Anti-D immunization can occur readily in D-negative recipients (Urbaniak, Transfusion 21:64, 1981).

**[0003]** The antigens of the RH blood group are carried by proteins coded by two genes, *RHD* and *RHCE,* that are located at chromosomal position 1p34.1 - 1 p36 (Cherif-Zahar, Hum. Genet. 86: 398, 1991; MacGeoch, Cytogenet. Cell Genet. 59:261, 1992) probably within less than a 450,000 base pair (bp) distance (Carritt, Hum. Mol. Genet. 6:843, 1997). Both genes encompass ten exons and their structures are highly homologous. The relative orientation of the genes, their distance, and the possibility of interspersed other genes were unknown (Flegel, Transfus. Med. 8:281, 1998). Very recently, Okuda et al. (Okuda, Biochem. Biophys. Res. Commun. 263:378, 1999) reported a sequence of about 11,000 bp, which was thought to represent the DNA segment between *RHD* and *RHCE.*

**[0004]** In whites, the vast majority of D-negative haplotypes is due to a deletion nf the *RHD* gene: This deletion spans the whole *RHD* gene, because *RHD*-specific sequences ranging from exon 1 to the 3' untranslated region are absent (Gassner, Transfusion 37:1020, 1997). The exact extent of the deletion was uncertain, leaving open the possibility that neighboring genes were also affected.

**[0005]** The identification of the *RHD* gene as the molecular basis of the D antigen allowed RhD phenotype prediction by DNA typing (Flegel, Transfus. Med. 8:281, 1998; Lo, Lancet 341:1147, 1993). However, since the structure of the prevalent D-negative haplotype is unknown, a specific detection of the *RHD* deletion remained impossible and the discrimination of $RHD^+/RHD^+$ homozygous from $RHD^+/RHD$ heterozygous individuals relied on indirect methods. This discrimination is of clinical interest in particular, because in D-negative mothers with an anti-D, the risk of an affected child is 100% with a $RHD^+/IRH^+$ father, but only 50% with a $RHD^+/RHD$ father.

**[0006]** Several indirect approaches have been applied to determine the zygosity: (i) a simple guess based on the phenotype is correct in about 95% of cases, (ii) determination of the D antigen density which can be confounded by factors such as the presence of the C antigen, and (iii) several methods involving the parallel quantitative amplification of *RHD*- and *RHCE*-specific sequences (Cossu, Electrophoresis 17:1911, 1996; Döscher, Infusionsther. Transfusionsmed. 26(suppl 1):31, 1999 (abstr.)). These elaborate techniques may not be practical in routine laboratories. In addition, several investigators identified polymorphisms in the *RICE* gene or neighboring sequences genetically linked to the lack of the *RHD* gene (Carritt, Hum. Mol. Genet. 6:843, 1997; Huang, Am. J. Hum. genet. 58: 133, 1996; Fujiwara, Hum. genet. 104:301, 1999; Onda, Gene 159:225,1995). This indirect approach relied on the linkage disequilibrium associating the *RHD* deletion with a polymorphism.

**[0007]** Furthermore, the utility of the *RHD* PCR is limited by the incomplete knowledge of presumably rare *RHD* positive alleles in RhD-negative. *RHD* positive alleles in RhD negative are caused by *RHD-CE-D* hybrid genes (Huang, Blood 88:2326-33, 1996; Faas, Transfusion 37:38-44, 1997, Faas, Transfusion 36:506-11, 1996), nonsense-mutations (Avent, Blood 89:2568-77, 1997), frameshifts (Andrews, Blood 92:1839-40, 1998; Cherif-Zahar Br. J. Haematol. 102:1263-70, 1998), or pseudogenes (Singleton, Blood 95:12-8, 2000). Such alleles are frequent in Africans (Faas, Transfusion 37: 38-44, 1997, Singleton, Blood 95:12-18, 2000) and Asians (Okuda, J. Clin. Invest. 100:373-9,1997) but rare in whites. Nevertheless, recent analyses (Avent, Blood 89:2568-77, 1997; Flegel, Transfus. Med. 8:281-302, 1998) suggested that even for whites these alleles are likely the leading cause of incorrect Rh phenotype prediction. Several observations in whites (Avent, Blood 89:2568-77, 1997; Hyland, Blood 84:321-4, 1994) indicated that these alleles clustered in the Cde and cdE haplotypes.

**[0008]** The most direct approach for analyzing the *RHD* locus on the molecular level would be PCR amplification spanning the *RHD* deletion site. Such an assay has, so far, not been available because the structure of the *RHD* locus in RhD positives and RhD negatives was incompletely understood.

**[0009]** Accordingly, the technical problem underlying the present invention was to provide means and methods for a reliable, nucleic acid based analysis of the Rhesus D locus. These means and methods should be, inter alia, suitable for the detection and/or discrimination of $RHD^+/RHD^+$ and $RHD^+/RHD^-$ individuals.

**[0010]** The solution to said technical problem is achieved by providing the embodiments characterized in the claims.

**[0011]** Thus, the invention relates to a nucleic acid molecule as characterised in the claims.

**[0012]** The *RHD* gene consists of a *RHD* 5' region homologous to genomic done HS469D22 (GenBank accession

number AL031284.9) bases 56,012 to 51,472; also represented by a nucleotide segment dubbed "stuffer fragment" (GenBank accession number AB029152) bases 7,716 to 11,005; the *RHD* promoter (GenBank accession number AJ252314) bases 1 to 1,246 (see Figure 11) and the *RHD* gene defined by the *RHD* cDNA (GenBank accession number X63097) bases 1 to 1,371 and by its intron sequences.

**[0013]** In accordance with the present disclosure, the term "nucleic acid molecular structure" comprises also any feasible derivative of the above referenced nucleic acid structure to which a nucleic acid probe may hybridize. In other words, the structure may be prepared by synthetic or semisynthetic means and thus consist of or comprise peptide nucleic acid. Said term also bears the meaning of a nucleic acid molecule.

**[0014]** In accordance with the present invention the term "identity" refers to the determination of sequence identity using suitable alignment programs, such as BLAST.

**[0015]** As has been pointed out above, the diagnostic analysis of *RHD* negatives on the molecular level has so far been hampered by the fact, that the overall structure of the *RHD*/*RHCE* loci was unknown. It has now been surprisingly found, that the two genes, *RHD* and *RHCE,* have opposite orientation and face each other with their 3' ends. It has further been found that the *RHD* gene is surrounded by two highly homologous *Rhesus boxes.* The physical distance between *RHD* and *RHCE* is about 30,000 bp and is filled with a *Rhesus box* and the *SMP1* gene. The breakpoints of the *RHD* deletion in the prevalent *RHD* negative haplotypes are located in the 1,463 bp identity region of the *Rhesus boxes.* Similar *RHD* deletion events may involve any other region within the highly homologous *Rhesus boxes.* Hence, a region of a breakpoint comprising an *RHD* deletion other than the common *RHD* deletion may be anticipated to occur anywhere within the *Rhesus boxes* as defined above.

**[0016]** The opposite orientation of the two *RH* genes explains the different character of hybrid genes in the MNS and RH blood group: The glycophorin genes encoding the MNS antigens occur in the same orientation (Onda, Gene 159: 225, 1995), and many recombinations may be explained as unequal crossing over resulting in single hybrid genes (Blumenfeld, Hum. Mutat. 6:1999, 1995). Based on the surprising findings referred to above, the events on the molecular level that lead to *RHD* negatives can now be more fully understood. In the *RH* locus, the inversely oriented sequences are unlikely to trigger unequal crossing over, and if this event occurred, no functional hybrid gene would result. The conclusion that unequal crossing over at the *RH* gene locus is unlikely may explain that most *RH* hybrid genes are of *RHD-CE-D* or *RHCE-D-CE* type and involve stretches of homologous DNA positioned *in cis* as noted previously (Wagner, Blood 91:2157, 1998). Currently, the *RH* gene system is the only well investigated gene locus where the two genes have opposite orientation, rendering it a model system for the evolution of neighboring, oppositely oriented genes that are frequent throughout genomes.

**[0017]** Based on the structure of the *RH* gene locus (Fig. 1), a parsimonious model for the *RHD* gene deletion event is proposed (Fig. 7). Although the applicant does not wish to be bound to theory, the following is believed with regard to the generation of RhD negative. The *RHD* deletion may be explained by unequal crossing over triggered by the highly homologous *Rhesus boxes* embracing the *RHD* gene. The hybrid-type *Rhesus box* of *RHD*-negatives arises, when a crossover leading to a deletion event involving a breakpoint region within the identity region of the upstream and downstream *Rhesus boxes* takes place. Thus, the hybrid *RHD* box is characterized by a 5' portion derived from the upstream *RHD* box fused to a 3' portion from the downstream *RHD* box. In one preferred embodiment the breakpoint region is 903bp long. The sequence of this preferred hybrid *Rhesus box* is depicted in figure 5. In the specific embodiments described in the examples, said 903 bp breakpoint region in *the Rhesus boxes* is located in a 1,463 bp stretch of 99.9% homology resembling a THE-1B human transposable element and a L2 repetitive DNA element (Fig. 4). Interestingly, the >60,000 bp DNA segment that is deleted in the *RHD* negative haplotype consisted only of and contained all sequences that are duplicated in the *RHD* positive haplotype.

**[0018]** The findings referred to herein above allow for the establishment of a number of easy to do or refined methods for the analysis of the genotype of an individual with regard to the *RH* gene locus. Examples of such methods are provided herein below.

**[0019]** While the molecular mechanism resulting in the prevalent *RHD* negative haplotype is now apparent, it is less clear how the much older duplication event gave rise to the structure of the *RH* genes in *RHD* positives. The duplication of the *Rhesus box* and the *RH* genes probably occurred as a single event, because the overall homology of the two *Rhesus boxes* is very similar to that of the *RH* genes. Without being bound by theory, it is tempting to speculate that the *RHD* duplication originate in causal connection with the insertion of the near full-length THE-1B transposon-like human element in duplicate. However, the open reading frame of the THE-1 B element probably was non-functional at the time of the duplication.

**[0020]** According to the present invention, the term "is representative of" relates to a nucleic acid molecular structure comprising all sequential and structural features to relate said structure to a group of molecular structures sharing said features. In the above preferred embodiment, said features give rise to the common *RHD* negative haplotype. In the present context this means preferably the deletion of the *RHD* gene encompassing the whole *RHD* gene and its 5' region, which are located between the upstream *Rhesus box* and the downstream *Rhesus box.*

**[0021]** In the present context this could also mean, for example, that all structures sharing a nonsense mutation,

missense mutation, splice site mutation, partial deletion, partial insertion, partial inversion or a combination thereof within the *RHD* gene, which terminates or obliterates the expression of a protein product of the *RHD* gene, are representative of the *RHD* negative haplotype.

**[0022]** The term "haplotype" relates to a series of linked alleles within a defined region on a single maternal or paternal chromosome.

**[0023]** The term "*RHD* positive haplotype" refers to any haplotype that comprises DNA sequences specific for the *RHD* gene.

**[0024]** In one embodiment the nucleic acid molecular structure is derived from a sample comprising an *RHD* positive haplotype that is serologically classified RhD negative.

**[0025]** In the context of the invention, the term "serologically classified RhD negative" describes a sample that has been tested for the presence of RhD antigen using, e.g., routine serological assays wherein the result of such assays was negative.

**[0026]** In a particularly preferred embodiment the sample that is classified RhD negative is obtained from a Caucasian population.

**[0027]** $Cde^s$, also known as $r^{rS}$, is a *RH* haplotype resembling *Cde* that was initially characterized as expressing antigen $e^s$ instead of antigen e, expressing antigen c, and expressing reduced and altered antigen C (Issitt, P.D. Aplied Blood Group Serology, Miami: Montgomery Scientific Publications, 1985, page 239). The molecular structure underlying this haplotype has recently been elucidated (Blunt, T., Daniels, G., and Carritt, B. Serotype switching in a partially deleted RHD gene. Vox Sang. 67:397-401, 1994; Faas, B.H.W., Becker, E.A.M., Wildoer, P., Ligthart, P.C., Overbeeke, M.A.M., Zondervan, H.A., von dem Borne, A.E.G.K., and van der Schoot, C.E. Molecular background of VS and weak C expression in blacks. Transfusion 37:38-44, 1997; Daniels, G.L., Faas, B.H., Green, C.A., Smart, E., Maaskant-van Wijk, P.A., Avent, N.D., Zondervan, H.A., von dem Borne, A.E., and van der Schoot, C.E. The VS and V blood group polymorphisms in Africans: a serologic and molecular analysis. Transfusion 38:951-958, 1998.): The $Cde^s$ haplotype contains a *RHD-CE-D* hybrid gene encoding for an antigen C immunoreactivity, in which exons 4 to 7 derived from *RHCE* and exon 3 has a *RHD* like structure but possesses a *RHCE* specific Thr at codon 152.

**[0028]** Several additional *RHD* positive alleles occurring in RhD negative individuals have previously been partly or fully characterized (Table 10). Three of these ten published *RHD* alleles represented *RHD-CE-D* hybrid alleles in which the *RHCE* specific stretch encompassed at least exons 4 to 7. For each of these three hybrid *RHD* alleles, alleles were found whose patterns would be compatible (Table 10). Out of the seven RhD negative patterns observed in the present study, six were compatible with such type of hybrid *RHD* allele. Seven out of ten published *RHD* alleles represented deletions, nonsense mutations or a pseudogene. None of these alleles occurred in this study, which may indicate that they are rare in whites.

**[0029]** The invention relates to a nucleic acid molecule being derived from the *RHD* gene comprising a single nucleotide substitution within the coding region of the *RHD* gene or within a 5' or 3' splice site, as characterised in the claims.

**[0030]** The term "a nucleic acid molecule derived from the *RHD* gens$^{n}$ is intended to mean that this nucleic acid molecule originates from the *RHD* gene but carries a mutation, deletion, insertion, substitution or duplication within the coding region, any of the splice sites or a non-coding region. Preferably, said nucleic acid molecule gives rise to an aberrant polypeptide.

**[0031]** In a further preferred embodiment said nucleotide substitution gives rise to a stop-codon at codon 16.

**[0032]** In a more preferred embodiment said substitution gene gives rise to an *RHD(W16X)* mutation.

**[0033]** In an additional more preferred embodiment said substitution is a G→A substitution at nucleotide position 48.

**[0034]** In a further preferred embodiment of the invention said nucleotide substitution gives rise to a stop codon at codon 330.

**[0035]** In a more preferred embodiment of the invention said substitution gives rise to a *RHD*(Y330X) mutation

**[0036]** In an even more preferred embodiment of the invention said substitution is a C→ G substitution at nucleotide position 985.

**[0037]** In another preferred embodiment of the invention said substitution gives rise to a missense mutation at codon 212.

**[0038]** In another preferred embodiment of the invention said substitution gives rise to a *RHD(G212V)* missense mutation.

**[0039]** In a more preferred embodiment of the invention said substituion is a G→T substitution at nucleotide position 635.

**[0040]** In a different preferred embodiment of the invention said substitution gives rise to a mutation within a 4 nucleotide sequence, a 6-nucleotide sequence or an 8-nucleotide sequence comprising the consensus splice site at the exon 8/intron 8 boundary.

**[0041]** In another more preferred embodiment of the invention said substituion give rise to a *RHD(G1153(+1)A)* mutation.

**[0042]** In an additional more preferred embodiment of the invention said substitution is a substitution at the 5' splice site intron 8 from AGgt to AGat.

**[0043]** In a further more preferred embodiment the nucleic acid molecular structure of the invention or a nucleic acid molecule being derived from the *RHD* gene correlates with a RhD-negative phenotype.

**[0044]** In another preferred embodiment of the invention said substitution gives rise to a mutation within a 4-nucleotide, a 6-nucleotide sequence or an 8-nucleotide sequence sequence comprising the consensus splice site of the exon 3/intron3 boundary.

**[0045]** In a further preferred embodiment of the invention said substitution gives rise to a *RHD(G486(+1)A)* mutation.

**[0046]** In an additional more preferred embodiment of the invention said substitution is a substitution at the 5' splice site intron 3 from ACgt to ACat.

**[0047]** In a further preferred embodiment of the invention said substitution gives rise to a mutation within a 4-nucleotide sequence, a 6-nucleotide sequence or an 8-nucleotide sequence comprising the consensus splice site of exon 9/intron 9 boundary.

**[0048]** In another preferred embodiment said substitution gives rise to a *RHD(K409K)* mutation.

**[0049]** In an additional more preferred embodiment of the invention said substitution is a substitution at the 5' splice site intron 9 from AGgt to AAgt.

**[0050]** In a more preferred embodiment of the invention the nucleic acid molecular structure of the invention or a nucleic acid molecule being derived from the *RHD* gene correlates with a $D_{el}$-phenotype.

**[0051]** In summary and referring to the above, *RHD* positive alleles can harbour single nucleotide substitutions leading to termination or reduction of the D-antigen expression. Using the improved detection methods disclosed in the present invention four *RHD* positive alleles in RhD negatives were found that had not been described previously. Two alleles, *RND*(W16X) and *RHD*(Y330X) harbored stop codons preventing the expression of the full RhD protein. In three alleles, splice site mutations were found that may prevent correct splicing and RhD expression.

**[0052]** These alleles typed *RHD* positive in all *RHD* PCR methods tested, and a correct antigen D prediction necessitates a specific detection of these alleles or of polymorphisms linked to these alleles.

**[0053]** Previously, the discrimination of *RHD* homozygotes from *RHD* heterozygotes was difficult. The prevalent *RHD* negative allele could not be detected specifically (Flegel, Transfus. Med. 8:281, 1998; Cossu, Electrophoresis 17:1911, 1996). The above defined mutation found in accordance with the present invention provides the basis for the detection of the prevalent *RHD* negative haplotypes, and hence true *RHD* genotyping is now feasible.

**[0054]** The invention additionally relates to a protein product of the *RHD* gene encoded by the nucleic acid molecule of the invention.

**[0055]** Preferably, the protein is in the same way post translationaly modified and has the same chemical structure as naturally occurring antigen.

**[0056]** Furthermore, the invention relates to an oligonucleotide hybridizing under stringent conditions to a portion of the nucleic acid molecules of the invention, wherein said portion comprises said (missense) mutation or said stop codon or to the complementary portion thereof as characterised in the claims.

**[0057]** In this embodiment of the invention, it is understood that the oligonucleotides hybridizes directly to the mutated sequence. The setting of stringent hybridization conditions is well described, for example, in Sambrook et al, "Molecular Cloning, A Laboratory Handbook" CSH Press, Cold Spring Harbor 1989 or Hames and Higgins, "Nucleic acid hybridization, a practical approach", IRL Press, Oxford (1985). Thus, the detection of the specifically hybridizing sequences will usually require hybridization and washing conditions such as 0.2 x SSC, 0.1 % SDS at 65°. As is well known, the length of the probe and the composition of the nucleic acid to be determined constitute further parameters of the stringent hybridization conditions. Preferably, the oligonucleotide is a deoxynucleotide. It is further preferred that the oligonucleotide comprises 12 to 50 nucleotides and more preferably 15 to 24 nucleotides.

**[0058]** Further, the invention relates to an antibody or an aptamer specifically binding to the protein product of the *RHD* gene of the invention.

**[0059]** The antibody may be tested and used in any serologic technique well known in the art, like agglutination techniques in tubes, gals, solid phase and capture techniques with or without secondary antibodies, or in flow cytometry with or without immunofluorescence enhancement.

**[0060]** The antibody of the invention may be a monoclonal antibody or an antibody derived from or comprised in a polyclonal antiserum. The term "antibody", as used in accordance with the present invention, further comprises fragments of said antibody such as Fab, $F(ab')_2$, Fv or scFv fragments; see, for example, Harlow and Lane, "Antibodies, A Laboratory Manual" CSH Press 1988, Cold Spring Harbor, N.Y. The antibody or the fragment thereof may be of natural origin or may be (semi) synthetically produced. Such synthetic products also comprise non-proteinaceous as semi-proteinaceous material that has the same or essentially the same binding specificity as the antibody of the invention. Such products may, for example, be obtained by peptidomimetics.

**[0061]** The term "aptamer" is well known in the art and defined, e.g., in Osbome et al., Curr. Opin. Chem. Biol. I (1997), 5-9 or in Stall and Szoka, Pharm. Res. 12 (1995), 465-483.

**[0062]** Furthermore, the invention relates to a method for testing for the presence of a nucleic acid molecule encoding a mutant Rhesus D antigen as characterized by the nucleic acid molecule of the invention in a sample comprising

hybridizing the oligonucleotide of the invention under stringent conditions to nucleic acid molecules comprised in the sample obtained from a human and detecting said hybridization.

**[0063]** Preferably, the method of the invention further comprises digesting the product of said hybridization with a restriction endonuclease or subjecting the product of said hybridization to digestion with a restriction endonuclease and analyzing the product of said digestion.

**[0064]** This preferred embodiment of the invention allows by convenient means, the differentiation between an effective hybridization and a non-effective hybridization. For example, if the wild type *RHD* gene comprises an endonuclease restriction site, the hybridized product will be cleavable by an appropriate restriction enzyme whereas a mutated sequence will yield no double-stranded product or will not comprise the recognizable restriction site and, accordingly, will not be cleaved. Alternatively, the hybridizing oligonucleotide may only hybridize to the mutated sequence. In this case, only a hybrid comprising the mutated sequence, but not the wild type sequence, will be cleaved by the appropriate restriction enzyme. The analysis of the digestion product can be effected by conventional means, such as by gel electrophoresis which may be optionally combined by the staining of the nucleic acid with, for example, ethidium bromide. Combinations with further techniques such as Southern blotting are also envisaged.

**[0065]** Detection of said hybridization may be effected, for example, by an anti-DNA double-strand antibody or by employing a labeled oligonucleotide. Conveniently, the method of the invention is employed together with blotting techniques such as Southern or Northern blotting and related techniques. Labeling may be effected, for example, by standard protocols and includes labeling with radioactive markers, fluorescent, phosphorescent, chemiluminescent, enzymatic labels, etc.

**[0066]** The invention additionally relates to a method for testing for the presence of a nucleic acid molecule encoding a mutant Rhesus D antigen as characterized by the nucleic acid molecule of the invention in a sample comprising determining the nucleic acid sequence of at least a portion of the nucleic acid molecule of the invention, said portion encoding said (missense) mutation, said stop codon or a breakpoint of said hybrid gene.

**[0067]** Preferably, the method of the invention further comprises, prior to determining said nucleic acid sequence, amplification of at least said portion of said nucleic acid molecular structure.

**[0068]** Furthermore, the invention relates to a method for testing for the presence of a nucleic acid molecule encoding a mutant Rhesus D antigen as characterized by the nucleic acid molecule of the invention in a sample comprising carrying out an amplification reaction using a set of primers that amplifies at least a portion of said sequence wherein at least one of the primers employed in said amplification reaction is the oligonucleotide of the invention. Moreover, in a further embodiment the method of the invention wherein at least one of the primers employed in said amplification reaction is the oligonucleotide of the invention.

**[0069]** Preferably, amplification is effected by polymerase chain reaction (PCR). Other amplification methods such as ligase chain reaction may also be employed.

**[0070]** In a preferred embodiment of the method of the invention said PCR is PCR-RFLP, PCR-SSP or long-range PCR.

**[0071]** Additionally, in another preferred embodiment of the invention the molecular weight of the amplification product is analyzed. Said analysis of the molecular weight utilizes standard techniques, such as agarose gel electrophoresis, SDS-PAGE, mass spectrometry such as MALDI-TOF for this purpose, which are well known to the person skilled in the art.

**[0072]** In one preferred embodiment of the method of the invention, said method detects *RHD* positive alleles comprising the following steps:

(a) isolating DNA from a blood sample or blood donor;
(b) hybridizing at least two oppositely oriented primers under stringent conditions to the DNA so as to carry out a PCR;
(c) amplifying the target sequence;
(d) separating the amplification products on a gel; and
(e) analyzing the amplicons.

**[0073]** With regard to specific conditions to be applied in the various steps, it is referred to the corresponding description herein above.

**[0074]** In a preferred embodiment the *RHD* positive alleles are derived from a serologically RhD negative population. In another preferred embodiment the RhD-negative sample is selected from a Caucasian population.

**[0075]** The method of the invention will result in an amplification of only the target sequence, if said target sequence carries the at least one mutation. This is because the oligonucleotide will, under preferably stringent hybridization conditions, not hybridize to the wild type sequence (with the consequence that no amplification product is obtained) but only to the mutated sequence. Naturally; primer oligonucleotides hybridizing to one or more as one, such as two mutated sequences may be employed in the method of the invention. The latter embodiment may be favorable in cases where combinations of mutations are tested for. It is important to note that not all or none of said mutations are necessarily missense mutations. This may be true for cases where other types of mutations occur in combination with the above missense mutations or with the above gene conversion.

**[0076]** Preferably, in the method of the invention said amplification or amplification reaction is or is effected by the polymerase chain reaction (PCR). Other amplification methods such as ligase chain reaction may also be employed.

**[0077]** Further, the invention relates to a method for testing for the presence of a protein product of the *RHD* gene of the invention in a sample comprising assaying a sample obtained from a human for specific binding to the antibody or aptamer or phage of the invention.

**[0078]** Testing for binding may, again, involve the employment of standard techniques such as ELISAs; see, for example, Harlow and Lane, "Antibodies, A Laboratory Manual" CSH Press 1988, Cold Spring Harbor.

**[0079]** In another preferred embodiment the invention relates to a method for testing for the presence of a protein product of the *RHD* gene encoding the nucleic acid molecule of the invention, comprising utilizing direct agglutination methods, indirect antiglobulin tests, monoclonal anti-D antibodies and adsorption/elution techniques.

**[0080]** Thus, the embodiment may comprise direct agglutionation with two monoclonal anti-D antibodies, alternatively indirect antiglobulin tests using a gel matrix comprising an oligoclonal anti-D antibody, in a further alternative using monoclonal anti-Rhesus antibodies in another alternative adsorption of polyclonal anti-D antibodies to red cells and elution using a chloroform technique. Further description of the methods is given in example 18.

**[0081]** Preferably, in the method of the invention said, sample is blood, serum, plasma, fetal tissue, saliva, urine, mucosal tissue, mucus, vaginal tissue, fetal tissue obtained from the vagina, skin, hair, hair follicle or another human tissue.

**[0082]** Furthermore, the method of the invention preferably comprises the step of enrichment of fetal cells. This enrichment may be achieved by using appropriate antibodies, lectins or other reagents specifically binding fetal cells or by any technique attempting the differential separation of maternal and fetal cells, like by density gradients. Also preferably, in said method fetal DNA or mRNA from maternal tissue like peripheral blood, serum or plasma may be extracted, advantageously according to conventional procedures.

**[0083]** In an additional preferred embodiment of the method of the invention, said nucleic acid molecule or proteinaceous material from said sample is fixed to a solid support.

**[0084]** Preferably, said solid support is a chip.

**[0085]** The advantages of chips are well known in the art and need not be discussed herein in detail. These include the small size as well as an easy access of computer based analysis of analytes.

**[0086]** Furthermore, the present invention relates to the use of the nucleic acid molecule of the invention for the analysis of a negative or a positive Rhesus D phenotype.

**[0087]** The analysis can be effected, for example, on the basis of the methods described herein above.

**[0088]** The invention also relates to the use of the nucleic acid molecule of the invention, or the protein product of the *RHD* gene of the invention for the assessment of the affinity, avidity and/or reactivity of monoclonal anti-D or anti-C antibodies or of polyclonal anti-D or anti-C antisera or of anti-globulin or of anti-human-globulin antisera or of preparations thereof.

**[0089]** Anti-C is a monoclonal antibody or polyclonal antiserum binding to antigen C.

**[0090]** The invention also relates to the use of cells, preferably red blood cells, from probands carrying the nucleic acid molecule of the invention for the assessment of the affinity, avidity and/or reactivity of monoclonal anti-D antibodies or of polyclonal anti-D antisera or of anti-globulin or of anti-human-globulin antisera or of preparations thereof.

**[0091]** Said preparations can be provided according to techniques well known in the art. Said preparations may comprise stabilisators such as albumins, further sodium azide, salt ions, buffers etc. The formulation of the preparation may have an influence on the binding characteristics of the antibodies, as is well known in the art.

**[0092]** For example, in a first step, the Rhesus D gene of a carrier or of a blood donor and its allelic status is analyzed and it is determined whether said gene comprises a mutation that was found in accordance with the present invention. In a second step, said mutation is correlated to a certain RhD antigen density on the surface of red blood cells. Conveniently, said correlation can be established by data provided in the present invention (such as mutations per se) and techniques that are well known in the art (see, e.g. Jones et al. 1996, Flegel and Wagner, 1996). In a third step, the features of an antibody or an antiserum such as reactivity, sensitivity, affinity, avidity, and/or specificity are determined with suitable blood group serological techniques preferably using red blood cells that were molecularly and with respect to the RhD antigen surface density characterized as described in step 2. Such data can be used, for example, in quality controls, standardization, etc.

**[0093]** The invention will be most useful for the characterization, standardization and quality control of monoclonal and polyclonal antisera, preferably anti-D monoclonals or antisera. Further, for example, anti-globulin and anti-human-globulin antisera can be characterized on the basis of the teachings of the present invention. An appropriately characterized anti-D monoclonal antibody can be conveniently used in RhD diagnostics. For example, a suitably characterized monoclonal antibody will be useful in determining the D antigen density on the surface of blood cells. Cut-off values for monoclonal antibodies useful in diagnosis can thus be established. This is important for the quality control of antibodies used in RhD diagnosis.

**[0094]** Thus, the invention also relates to a method for the characterization of monoclonal antibodies or polyclonal antisera or of a preparation thereof, said method comprising

(a) testing the nucleic acid of sample of a proband for the presence of a mutation as defined in accordance with the invention;
(b) correlating, on the basis of the mutation and the allelic status of the *RHD* gene, the nucleic acid with the density of the protein product of the *RHD* gene on the surface of red blood cells of said proband;
(c) reacting said monoclonal antibodies or polyclonal antisera or said preparation thereof with a cell carrying the protein product of the *RHD* gene on its surface;
(d) characterizing said monoclonal antibodies or polyclonal antisera or said preparation thereof on the basis of the results obtained in step (c).

**[0095]** As regards the term "allelic status", this term describes the possibilities that the *RHD* alleles in a proband are present in a homozygous, heterozygous or hemizygous state. Also comprised by this term is the possibility that the two alleles carry two different mutations (including the conversion) defined herein above.

**[0096]** In a preferred embodiment of the method of the invention, said characterization comprises the determination of reactivity, sensitivity, avidity, affinity, specificity and/or other characteristics of antibodies and antisera.

**[0097]** Furthermore preferred is a method wherein said cell carrying the protein product of the *RHD* gene on its surface is a red blood cell.

**[0098]** The invention also relates to a method for determining whether a patient in need of a blood transfusion is to be transfused with RhD negative blood from a donor comprising the step of testing a sample from said patient for the presence of one or more nucleic acid molecules of the invention, wherein a positive testing for two different of said nucleic acid molecules is indicative of the need for a transfusion with Rh negative blood. Alternatively, a positive testing indicating the concomitant presence of two identical copies of one of said nucleic acid molecular structures or nucleic acid molecules is indicative of the need for a transfusion with Rh negative blood.

**[0099]** Alternatively, a negative testing for the presence of the nucleic acid molecular structure or nucleic acid molecule representative of the common *RHD* negative haplotype with or without a negative testing for one or more nucleic acid molecular structures or nucleic acid molecules representative of the other *RHD* negative nucleic acid molecular structures or nucleic acid molecules of this invention permits the transfusion of blood that is typed as RhD positive. The invention has important implications for devising a transfusion therapy in humans. For example, it can now be conveniently tested whether the patient actually needs a transfusion with a RhD negative blood or whether such precautions need not be taken.

**[0100]** Also described herein is a method for determining whether blood of a donor may be used for transfusion to a patient in need thereof comprising the step of testing a sample from said donor for the presence of one or more of said nucleic acid molecules of the invention, wherein a negative testing for the nucleic acid molecular structures representative of the common *RHD* negative haplotype with or without a negative testing for one or more nucleic acid molecular structures or nucleic acid molecules representative of the other *RHD* negative haplotypes of this invention excludes the transfusion the donor's blood to a patient that is typed as RhD negative.

**[0101]** Also described herein is a method for determining whether the blood of a donor may be transfused to a patient typed as RhD negative comprising the step of testing a sample from said donor for the presence of one or more nucleic acid molecules of the invention, wherein a positive testing for two different of said nucleic acid molecules is indicative of the possibility to transfuse the donor's blood to a patient typed as RhD negative.

**[0102]** Alternatively, a positive testing indicating the concomitant presence of two identical copies of one of said nucleic acid molecules is indicative of the possibility to transfuse this donor's blood to a patient that is typed as RhD negative.

**[0103]** The samples referred to in the above recited methods may be samples that are referred to throughout the specification, such as blood, serum, etc.

**[0104]** As regards the guidelines for transfusing a patient on the basis of any of the above recited methods, the utmost care must be taken that suboptimal transfusion policy is avoided. The risk factor is always to be considered by the physician in charge. In all cases, the potential risk for the patient is to be minimized.

**[0105]** The invention also relates to a method for assessing of the risk of a RhD negative mother of conceiving or carrying an RhD positive fetus or of the risk of a mother having an anti-D titer of conceiving or carrying a fetus at risk to develop hemolytic disease of the newborn comprising assessing a sample obtained from the father of the fetus for the presence of one or more of said nucleic acid molecular structures or nucleic acid molecules of the invention, wherein a negative testing for nucleic acid molecular structures or nucleic acid molecules representative of the common *RHD* negative haplotype with or without a negative testing for one or more nucleic acid molecular structures or nucleic acid molecules representative of the other *RHD* negative haplotypes of this invention is indicative for a high risk of conceiving an RhD positive fetus.

**[0106]** In a preferred embodiment of the method of the present invention said nucleic acid molecular structure carries mutations or deletions.

**[0107]** Also described herein is a method for determining whether the father is RhD negative comprising the step of testing a sample from the father for the presence of one or more nucleic acid molecular structures or nucleic acid molecules of the invention, wherein a positive testing for two different of said nucleic acid molecular structures or nucleic

acid molecules is indicative of the father being RhD negative.

**[0108]** Alternatively, a positive testing indicating the concomitant presence of two identical copies of one of said nucleic acid molecular structures or nucleic acid molecules representative of *RHD* negative haplotypes is indicative of the father being RhD negative.

**[0109]** Furthermore, also described herein is a method for assessing the possibility or likelihood of a man being the father of a child by assaying a sample obtained from said man for the presence of one or more of said nucleic acid molecular structures or nucleic acid molecules of the invention, wherein the test results are used to determine the homozygosity for, the heterozygosity for or the absence of any nucleic acid molecular structures or nucleic acid molecules representative of the *RHD* negative haplotype of the present invention used to infer the possibility or likelihood of said man being the father of the child.

**[0110]** The preparation may be a diagnostic or pharmaceutical preparation.

**[0111]** The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier and/or diluent. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose.

**[0112]** Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents such as interieukins or interferons depending on the intended use of the pharmaceutical composition.

**[0113]** Also described herein is a method of treating a pregnant woman being Rhesus D negative wherein the fetus does not carry two nucleic acid molecular structures or nucleic acid molecules of the invention or is not homozygous for any nucleic acid molecular structure or nucleic acid molecule of the invention, comprising administering anti-D to said woman.

**[0114]** Pregnant women may be currently treated with an anti-D prophylaxis, when a Rhesus negative mother carries a RhD positive fetus. Also described herein is the discrimination of an anti-D prophylaxis requirement depending on the status of the mother's and/or the fetus' possessing a RhD protein of the invention. One or more of the RhD proteins may be prone to immunization of their carriers and, hence, would be indicative for the therapy of the mother. Similarly, one or more RhD proteins, when carried by the fetus, may be known to be of low immunogenicity to the mother and, hence, would be indicative for the omission of anti-D prophylaxis in difference to current clinical therapy.

**[0115]** The administration can be effected by standard routes and doses which can be defined by the attending physician; Mollison, 1993. Preferably, a monoclonal anti-D or combinations/mixtures of monoclonal anti-Ds is/are administered in doses of 50 $\mu$g to or exceeding 500 $\mu$g anti-D antibody/antisera for intravenous or intramuscular administration (Bowman, 1998). For the quality control of these anti-D antibodies/antisera, the results and methods provided by the present invention may be advantageously employed.

**[0116]** Also described herein is the use of a phage, aptamer, monoclonal antibody or a polyclonal antisera or a preparation thereof as characterized in the present invention for determination of the protein product of the *RHD* gene.

**[0117]** It is further described herein that said determination of the protein product of the *RHD* gene is effected in connection with blood group typing.

**[0118]** Furthermore, the invention relates to a preparation comprising the antibody or aptamer or phage of the invention.

**[0119]** The present invention also relates to a method of identifying an antibody $V_H$ or $V_L$ chain or a combination thereof or an aptamer specifically binding to a protein product of the *RHD* gene of the invention comprising

(a) contacting the protein product of the *RHD* gene of the invention with a phage library displaying $V_H$ or $V_L$ chains or combinations thereof on the surface of the phage or with aptamers;
(b) identifying phage or aptamers that bind to said protein product of the *RHD* gene; and optionally
(c) repeating steps (a) and (b) one or more times.

**[0120]** The preparation of phage library and the screening/identification of desired antibody (chains) per se is well known in the art and reviewed, for example, in Winter et al., Annu. Rev. Immunol. 12 (1994), 433-455 and references cited therein. Also, aptamers can be prepared and cloned in phage according to conventional protocols. Whereas single $V_H$ or $V_L$ chains may be identified by the method of the invention as binding to the protein product of the *RHD* gene of the invention, it is preferred to identify $V_H$-$V_L$ combinations expressed by the phage because this situation resembles

the situation of natural antibody binding. By repeating steps (a) and (b) one or more times, better binding specificities may be identified. Protocols for the optimization of binding properties such as affinities, including elution steps for removing bound phage, are well established in the art. For example, once a $V_H$ chain with a convenient binding capacity has been found, $V_L$ chains may be identified that significantly improve the binding capacity of the antibody, e.g. by replacing the $V_L$ chain that was associated with the $V_H$ chain in the first selection step with a more suitable $V_L$ chain.

**[0121]** The invention also relates to a method of identifying a monoclonal antibody specifically binding to a protein product of the *RHD* gene of the invention comprising

(a) contacting the protein product of the *RHD* gene of the invention with one or more monoclonal antibodies;
(b) identifying monoclonal antibodies that bind to said protein product of the *RHD* gene; and optionally
(c) repeating steps (a) and (b) one or more times.

**[0122]** The invention also relates to a method of identifying an antibody $V_H$ or $V_L$ chain or a combination thereof or an aptamer specifically binding to a protein product of the *RHD* gene of the invention comprising

(aa) contacting said protein product of the *RHD* gene and
(ab) a normal D polypeptide
wherein the normal D polypeptide is present in a molar mass that is higher, equal or less than the protein product of the *RHD* gene of (aa) with a phage library displaying $V_H$ or $V_L$ chains or combinations thereof on the surface of the phage or with aptamers;
(b) identifying phage or aptamers that bind to said protein product of the *RHD* gene of (a); and optionally
(c) repeating steps (a) and (b) one or more times.

**[0123]** Particularly preferred in step (ab) is that the molar mass of the normal D polypeptide is higher than that of the protein product of the *RHD* gene of (aa).

**[0124]** In the case that only one round of selection is employed for the identification (i.e. when step (c) does not apply), it is preferred that the number of protein product of the *RHD* gene of (aa) is in molar excess over the number of phage particles. The preferred embodiments of the method of identifying an antibody $V_H$ or $V_L$ chain or of a combination thereof or of an aptamer described hereinbefore equally apply to this embodiment of the invention.

**[0125]** The invention also relates to a method of identifying a monoclonal antibody specifically binding to a protein product of the *RHD* gene of the invention comprising

(aa) contacting the protein product of the *RHD* gene and
(ab) a normal D polypeptide
wherein the normal D polypeptide is present in a molar mass that is higher, equal or less than the protein product of the *RHD* gene of (aa) with one or more monoclonal antibodies;
(b) identifying monoclonal antibodies that bind to said protein product of the *RHD* gene of (aa); and optionally
(c) repeating steps (a) and (b) one or more times.

**[0126]** Preferably, the protein product of the *RHD* gene is exposed on the surface of a cell. An appropriate surface is the surface of an erythrocyte. However, other host cells may be transfected with a vector suitable for expression of the protein product of the *RHD* gene of the invention and express the same on their surface. Antibodies may also bind to recombinant proteins of or parts of proteins of D antigen and purified proteins.

**[0127]** It is further preferred that the polypeptide or host cell is affixed to a solid support. Suitable examples for solid supports are microtiter plates or beads.

**[0128]** In an additionally preferred antibody, subsequent to step (b) or (c), the following step is carried out:

(d) identifying the amino acid sequence of the $V_H$ or $V_L$ chains and/or identifying the nucleic acid sequences encoding said amino acid sequence.

**[0129]** The identification of the amino acid/nucleic acid sequences can be effected according to conventional protocols; see, e.g., Sambrook et al., loc. cit.

**[0130]** Hence and in summary, the present invention provides means and methods for the detection of *RHD* haplotypes, comprising presumably rare *RHD* positive alleles in serologically RhD negative populations. Latter alleles, harbouring *RHD* sequences and therefore determined as *RHD*-positive, can comprise either *RHD*/*RHCE* hybrid genes, stop codons, splice site mutations or gene deletions, that terminate or reduce the RhD antigen expression. Carrying out the improved detection methods of the invention, it was surprisingly found, that several samples, determined as RhD negative in routine serology, could be identified having *RHD* positive alleles. Furthermore, some of those samples were even RhD

antigen positive when performing a detection assay based on adsorbtion and elution, indicating that the molecular basis for the *RHD* positive alleles in RhD negatives is more heterogenous than anticipated. Advantageously, the disclosure content of the present invention now provides new and practicable nucleic acid amplification techniques to determine whether *RHD* specific sequences cause RhD positive or RhD negative phenotypes.

**[0131]** In a particularly preferred embodiment the method of present invention, wherein, in the case that only one round of selection is employed for the identification, the number of protein molecules of the *RHD* gene of (a) is in molar excess over the number of phage particles.

**[0132]** Moreover, the present invention relates to the use of cells, preferably red blood cells comprising the protein product of the *RHD* gene of the present invention, from probands for the assessment of the affinity, avidity and/or reactivity of monoclonal anti-D antibodies of the present invention or of polyclonal anti-D antisera or of anti-globulin or of anti-human-globulin antisera or of preparations thereof.

**[0133]** According to the present invention the term "polymorphism" relates to the existence in a population of more than one genetic structure or a gene of a haplotype or of a DNA segment. Nevertheless, sometimes such a genetic polymorphism does not always result in a differing phenotype, but may only be detected at the genetic level.

**[0134]** Furthermore, the invention relates to a kit comprising

(a) the oligonucleotide of the invention; and/or
(b) the antibody of the invention;
(c) the aptamer of the invention; and/or
(d) the phage of the invention;
(e) a pair of primers useful for carrying out the amplification reaction of the invention.

**[0135]** Parts of the kit can be packaged individually in vials or in combination in containers or multicontainer units. The kit of the present invention may be advantageously used for carrying out the method of the invention and could be, inter alia, employed in a variety of applications referred to above. The manufacture of the kits follows preferably standard procedures which are known to people skilled in the art.

**[0136]** The figures show

**Figure 1** Schematic structure of the *RH* gene locus. The positions and orientations of the genes and the *Rhesus boxes* are indicated by open arrows and triangles, respectively (Panel A). The exons are shown as vertical bars and their exon number is indicated. The two *RH* genes have opposite orientation, face each other with their 3' ends, and are separated by about 30,000 bp. A third gene, *SMP1,* has the same orientation as *RHD* and is positioned in between *RHD* and *RHCE.* The *RHD* gene is flanked on both sides by the two highly homologous *Rhesus boxes* (b). All exons are shorter than 200 bp with the exception of the *RHD* and *SMP1 3'* terminal exons. Data used to establish this structure (Panel B) include the extension of genomic sequences represented in the cDNAs (horizontal arrows), identities and homologies to genomic clones (bar a: identity with dJ465N24; b: homology of *RHD* to dJ469D22; c: homology of *RHD* 3' part to dJ465N24; d: identity with dJ469D22). The positions of three bridging PCR reactions are indicated. They correct position of a nucleotide stretch previously reported by Okuda et al. (Okuda, Biochem. Biophys. Res. Commun. 263:378, 1999) as "spacer" sequence between *RHD* and *RHCE* is indicated by the bar labeled s.

**Figure** 2 Chromosomal organization of the DNA regions located 5' to the *RHD* and *RHCE* genes. The proposed structure of the *RHCE* and *RHD* 5' flanking regions is depicted (Panel A). A total of 4,941 bp immediately 5' of the ATG start codons are homologous between the *RHCE* and *RHD* genes (vertically hatched bars). No homology is present further beyond this homology region (diagonally hatched bars). Two genomic clones, dJ469D22 and dJ465N24, were utilized for primer design. DJ469D22 comprises the full length of the depicted *RHCE* region, whereas dJ465N24 extends only 466 bp into the homology region. The positions of several PCR primers are indicated (a, rey14a; b, rend32; c, rey15a; d, re014; e, re011d). This proposed structure is supported by several PCR reactions (panel B). Forward priming was done with primer a (*RHCE* specific, lane 1 - 3), primer b (*RHD* specific, lane 4 - 6), and primer c (*RHCE* and *RHD* homology region, lane 7 - 9). Amplicons were lacking for primer a with *RHD* specific reverse primer e (lane 2) and for primer b with *RHD* negative DNA (lane 6). The other seven PCR reactions yielded amplicons of the predicted sizes in accordance with the genomic structure shown in panel A.

**Figure 3** Chromosomal organization of the *SMP1* gene. The *SMP1* gene has seven exons. The positions and approximate sizes of the introns are shown. The start of the published cDNA (GenBank accession number AF081282) is separated by 15 nucleotides from the downstream *Rhesus box.* Exon 1 contains only 5'

untranslated sequence, the *SMP1* start codon is located in exon 2. Exon 7 contains 16 codons and 1,656 bp 3' untranslated sequences and is contiguous with the 3' untranslated sequence of *RHCE* exon 10.

**Figure 4** Chromosomal organization of the *Rhesus boxes.* The physical extension of the upstream *Rhesus box* (5' to *RHD*) is 9,145 bp (black bar). About 63% of the boxes' nucleotide sequence consists of repetitive DNA; the types of the repeat families are indicated. The overall homology between the upstream and downstream *Rhesus box* is 98.6%, but within an 1,463 bp identity region (horizontal arrows), there is only a single 4 bp insertion (double vertical line). A CpG-island (double-headed arrow) is located at the 3' end and is in the downstream *Rhesus box* (3' to *RHD*) adjacent to the *SMP1* promoter.

**Figure 5** *RHD* gene deletion in the Rh negative haplotypes. Three 3,100 bp segments of the *Rhesus boxes* are shown. The upper line indicates the nucleotide sequence of the upstream *Rhesus box* in D-positives, the lower line the nucleotide sequence of the downstream *Rhesus box* in D-positives. The middle line gives the nucleotide sequence of the single *Rhesus box* carried by Rh negatives. Asterisks denote identical nucleotides. The *RHD* deletion occurred in a 903 bp segment of absolute identity that was part of a 1,463 bp identity region. The positions of primers rez7 and mb31 is shown (m indicates mismatch). *Pst*I restriction sites are indicated by carets (^). The three *Rhesus boxes* are deposited at EMBL under accession numbers AJ252311 (upstream *Rhesus box),* AJ252312 (downstream *Rhesus box*), and AJ252313 (hybrid *Rhesus box).*

**Figure 6** Two technical procedures for specific detection of the *RHD* deletion in the common *RHD* negative haplotypes. A long-range PCR amplification with primers located in non-*Rhesus box* sequences (Panel A) and PCR-RFLP with primers located in the *Rhesus boxes* are shown (Panel B). The deduced genotypes are indicated. The primers of the long-range PCR were located 5' of the upstream *Rhesus box* (primer rez4) and in *SMP1* exon 1 (primer sr9). *RHD* negative haplotypes were detected specifically (Panel A, lane 1 - 6). DNA homozygous for the *RHD* gene was negative, because the PCR cannot amplify the 70,000 bp DNA stretch of the *RHD* gene. For the PCR-RFLP method, the PCR amplicons (primer rez7 and mb31) were digested with *Pst*I. In D-negatives, there are three *Pst*I sites in the amplicon (see Fig. 5) resulting in fragments of 1,888 bp, 564 bp, 397 bp, and 179 bp (lane 1 to 3). The downstream *Rhesus box* of D-positives lacks one *Pst*I-site resulting in fragments of 1,888 bp, 744 bp, and 397 bp (lane 7 to 9). $RHD^{+}/RHD^{-}$ heterozygotes show both fragments of 744 and 564 bp (lane 4 to 6). The 564 bp fragment appears weaker because heterodimers are not cut by *Pst*I. Primer mb31 does not amplify the upstream *Rhesus box* of D-positives.

**Figure 7** Model of the proposed mechanism causing the prevalent *RHD* negative haplotypes in whites. The physical structure of the *RHD* and *RHCE* gene locus is depicted (panel A). An unequal crossing over between the upstream and downstream *Rhesus boxes* can be triggered by their high homology (panel B). The breakpoint region in the *Rhesus boxes* was found to be of 100% homology for 903 bp (see Fig. 5). Resolving the crossed over chromosome yields the *RH* gene structure of the extant *RHD* negative haplotype (panel C).

**Figure 8** DNA sequence of the hybrid *Rhesus box* of *RHD* negatives.

**Figure 9** DNA sequence of the upstream *Rhesus box* of D-positives.

**Figure 10** DNA sequence of the downstream *Rhesus box* of D-positives.

**Figure 11** DNA sequence of the *RHD* promoter. The last three nucleotides represent codon 1 of the *RHD* gene.

**Figure 12.** *Cde^s* breakpoint region in *RHD* intron 3. The nucleotide sequence of a part of the intron 3 of *Cde^s, RHD* and *RHCE* 2,938 to 3,636 bp 3' of the exon 3/intron 3 junction is shown. The human DNA sequence from clone RP3-469D22 on chromosome 1p35.1-36.13 containing the 5' part of the gene for *RHCE* (GenBank accession number AL031284) was taken as reference; numbers indicate the position in this sequence relative to the first base of intron 3 in the *RHCE* gene. The corresponding *RHD* gene sequence derives from GenBank accession number AL139426. Nucleotides indicating *RHD* or *RHCE* origin of the *Cde^s* sequences are highlighted. A 154 bp DNA stretch comprising the breakpoint region of *Cde^s* is indicated by asterisks.

**Figure 13.** *Cde^s* breakpoint region in *RHD* intron 7. The nucleotide sequence of a part of the intron 7 of *Cde^s*, *RHD* and *RHCE* about 2,726 to 3,719 3' of the exon 7/intron 7 junction is shown. The human DNA sequence from clone RP3-469D22 on chromosome 1p35.1-36.13 containing the 5' part of the gene for *RHCE* (GenBank accession number AL031284) was taken as reference; numbers indicate the position in this sequence relative to the first base of intron 7 in the *RHCE* gene. The corresponding *RHD* gene sequence derives from GenBank accession number AL139426. Nucleotides indicating *RHD* or *RHCE* origin of the *Cde^s* sequences are highlighted. A 666 bp DNA stretch comprising the breakpoint region of *Cde^s* is indicated by asterisks.

**Figure 14.** Specific detection of *Cde^s* by PCR-SSP. A PCR-SSP detecting the 3' breakpoint region of *Cde^s* in intron 7 is shown. Both, a *RHD* negative sample (lane 1, ccddee) and a normal *RHD* positive sample (lane 2, ccD.EE) yield the 434 bp control product only, which is derived from the HGH gene. In contrast, a *Cde^s* sample (CcddEe, lane 3) yields the 338 bp specific product, which is derived from the breakpoint region in intron 7, and in addition the 434 bp control fragment. This reaction is specific for *Cde^s*; the two partial D phenotypes $D^{rva}$ (lane 4) and $D^{III}$ type IV (lane 5) do not yield a specific product. The reaction also detects *Cde^s* specifically, if *Cde^s* occurs *in trans* to other *RHD* alleles, like in a *RHDΨ*/*Cde^s* sample (lane 6).

**Figure 15.** *RHD* PCR-SSP for routine DNA typing. The PCR is performed as a modular system consisting of two multiplex reactions, an intron 4/exon 7 multiplex PCR-SSP (Panel A) and an intron 7 PCR enhanced by specific detection of *RHD*(W16X) and *RHDΨ* (Panel B). Results are shown for a normal D positive sample (lane 1), a normal D negative sample (lane 2), several rare D negative samples (lanes 3 to 6) and major D positive *RHD* variants (lanes 7 and 8). Standard D positive and D negative samples and D categories IV and VI are recognized in reaction A. *RHO-CE(8-9)-O* is detected in reaction B by the absence of the intron 7 band. The presence of RHD(W16X) and *RHDΨ* is also detected in reaction B. Band size is Panel A, control, 434 bp (HGH gene); intron 4, 226 bp; exon 7, 123 bp; Panel B, control, 659 bp (chromosome 1 genomic sequence about 90,000 bp 5' of *Rhesus box*); intron 7.390 bp; *RHD*(W16X), 248 bp; *RHDΨ,* 154 bp. The internal control amplicons, which were devised to be larger than the specific amplicons, may be suppressed because of competition, if a specific product is amplified.

**[0137]** The examples illustrate the invention:

**Example 1: Blood samples and DNA isolation**

**[0138]** EDTA- or citrate-anticoagulated blood samples were collected from white blood donors and characterized as D negative in routine typing including an antiglobulin test with anti-D (Wissenschaftlicher Beirat der Bundesärztekammer; Paul-Ehrlich-Institut. Richtlinien zur Blutgruppenbestimmung und Bluttransfusion (Hämotherapie). Köln: Deutscher Ärzte-Verlag; 1996; Wagner, Infusionsther Transfusionsmed 22:285-90, 1995). If necessary, samples were collected at random for specific CcEe phenotypes. A total of 314 ccddee, 433 Ccddee, 271 ccddEe, 19 CcddEe, 24 CCddee, 1 CcddEE and 6 ccddEE samples were tested. DNA was isolated by a modified salting-out procedure as described in Gassner et al., Transfusion 37; 1020, 1997.

**Example 2: Molecular work-up**

**[0139]** All samples were tested by PCR-SSP for the presence of four different *RHD* specific polymorphisms located in the *RHD* promoter, intron 4, exon 7 and the 3' untranslated region of exon 10. 48 samples with at least one positive PCR reaction were detected (Table 5). Those samples were further investigated for the presence of *RHD* specific polymorphisms in exon 3, exon 4, exon 5, exon 6, exon 7, intron 7 and exon 9. Twenty-six samples showed one of eight distinct PCR patterns involving a mixture of positive and negative reactions (Table 6). Twenty-two samples were positive for all *RHD* specific polymorphisms investigated and were assigned to eight *RHD* alleles by *RHD* specific sequencing of the ten *RHD* exons from genomic DNA (Table 7). For each PCR pattern and each *RHD* allele, one sample was serologically investigated. The phenotypes were determined to represent weak D, partial D, and $D_{el}$, or confirmed as serologically D negative by adsorption/elution (Table 6 and 7).

**Example 3: DNA database searches and analysis**

**[0140]** The GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) and the chromosome 1 database of the Sanger Center (htip://www.sanger.ac.uk/cgi-bintnph-Biast_Server.html) were searched with cDNA sequences representative of *RHD* (RhXIII, accession number X63097) and *RHCE* (RhVI, X63095) using the BLAST program. The 84,810 bp genomic

clone dJ469D22 (GenBank accession number AL031284), the 129,747 bp genomic clone dJ465N24 (GenBank accession number AL031432) and the 2,234 bp *SMP1* cDNA (GenBank accession number AF081282) were identified. dJ469D22 represented a major fragment of the *RHCE* gene, starting 33,340 bp 5' of the *RHCE* start codon and ending 1,142 bp 3' of exon 9. In dJ465N24, an internal stretch of 1,418 bp located between position 120,158 and 121,568 was 96% homologous to the 3' end of the *RHD* cDNA. The 3' end of the *SMP* cDNA was complementary to the 3' end of the *RHCE* cDNA with an overlap of 58 bp.

**Example 4: PCR**

**[0141]** If not mentioned otherwise, PCR reactions were done with 60°C annealing, 10 min extension at 68°C and denaturation at 92°C using the expand long template or the expend high fidelity PCR systems (Boehringer Mannheim, Mannheim, Germany) and the listed primers (Table 1). Three PCR reactions were used to bridge gaps in the 3[i] flanking regions of the *RH* genes. PCR 1 was done using primers rea7 and rend31 (PCR 2, rend32, sf1c; PCR 3 , rea7, sf3). The structure of the 5' flanking regions was confirmed with PCR amplifications involving sense primers rend32, rey14a, rey15a and antisense primers re011d and re014. Intron 9 size was estimated to be about 9,000 bp based on PCR amplifications using rb10b and rr4 for *RHD* (re96 and rh7 for *RHCE).*

**Example 5: Nucleotide sequencing**

**[0142]** Nucleotide sequencing was performed with a DNA sequencing unit (Prism BigDye terminator cycle-sequencing ready reaction kit; ABI 373A, Applied Biosystems, Weiterstadt, Germany).

**Example 6: Characterizing the *RH* gene locus**

**[0143]** A physical structure of the *RH* genes' locus was derived (Fig. 1). This structure was deduced from the following considerations: (i) 3' flanking regions. The 3' flanking region of *RHD* was highly homologous to the 3' part of dJ465N24 (Fig. 1B, region c). This horology continued beyond the end of the *RHD* cDNA and extended for at least 8,000 bp as proven by the fact that it was possible to obtain PCR amplicons (Fig. 1B, PCR 1). Sequences homologous to the 3' part of dJ465N24 were neighboring to the 5' region of the *SMP1* gene (Fig. 1B; PCR 2). The 3' end of the *SMP1* gene occurred immediately adjacent to the *RHCE* gene as indicated by the complementarity of the 3' ends of the respective, cDNAs and confirmed by PCR (Fig. 1B. PCR 3). Further details of the *RHD* 3' flanking region (Rhesus *box*) and the *SMP1* gene are described below. (ii) **5' flanking regions.** dJ469D22 comprised 33,340 bp 5' franking region of *RHCE.* For *RHD,* a 466 bp homology between the 3' end of dJ465N24 and dJ469D22 indicated that dJ465N24 might represent the 5' flanking sequence of *RHD.* This assumption was proven by PCR (Fig. 2). (iii) **Analysis of YAC 38A-A10.** DNA from the YAC 38A-A10 (UK HGMP resource centre, Cambridge, UK) was isolated after a single growth phase by standard methods (http://hdklab.wustl.edu/lab_manuavyeast). It was confirmed that this YAC contained *RH* DNA. Furthermore, shotgun cloning experiments indicated that some of its insert probably derived from the X chromosome (data not shown). This YAC had been known to contain *RHCE* exons 2 to 10 and *RHD* exons 1 to 10 (Carritt, Hum. Mol. Genet. 6:843, 1997) and was thus expected to contain the DNA segments interspersed between *RHD* and *RHCE.* The presence of DNA segments representative of different parts of the *RH* locus in this YAC was observed (Table 2). The results were concordant with the proposed structure of the *RH* locus shown in Fig. 1, Panel A.

**Example 7: Identification of *RHD* specific sequences in the *RHD* promoter**

**[0144]** About 2,000 bp *RHD* promoter sequence was established by chromosomal walking (GenomeWalker kit, Clontech, Heidelberg, Germany). D-positive and D-negative samples were amplified using primers re04 and re11d (Table 1) and *RHD-* and *RHCE*-specific sequences established for 1,200 bp 5' of the start codon by sequencing with internal primers. A short deletion in the *RHD* gene was identified and used to develop the *RHD*-specific primer re011d. The 1,200 bp sequence including the *RHD* promoter has been deposited at EMBL under accession no. AJ252314.

**Example 8: Characterization of *Rhesus boxes***

**[0145]** Two DNA segments of about 9,000 bp, located 5' and 3' of the *RHD* gene, were highly homologous, had identical orientation, and were designated *"Rhesus boxes"* (Fig. 4). The *Rhesus boxes* were amplified and sequenced using internal primers in two overlapping fragments using PCR primer pairs rez4/rend31 and rend32/re011d (upstream *Rhesus box*), rea7/rend31 and rend32/sr9 (downstream *Rhesus box*), and rez4/rend31 and rend32/sr9 (hybrid *Rhesus box* of *RHD*-negative). The upstream *Rhesus box* (5' of *RHD*) was about 9,142 bp long and ended about 4,900 bp 5' of the *RHD* start codon. The downstream *Rhesus box* (3' of *RHD*) was 9,145 bp long and started 104 bp after the *RHD*

stop codon. The *Rhesus boxes* exactly embraced the part of *RHD* with homology to *RHCE*. The central portion of both *Rhesus boxes* contained an almost complete remnant of a transposon-like human element (THE-1B). The single open reading frame usually found in the THE-1B element was, however, abolished due to several nucleotide aberrations occurring in both *Rhesus boxes* in parallel, including a nonsense mutation in codon 4. While there was overall 98.6% homology between both *Rhesus boxes,* a 1,463 bp "identity region" located between positions 5,701 and 7,163 was completely identical with the single exception of a 4 bp T insertion in a poly T tract.

**Example 9: Evaluation of the genomic structure of *SMP1***

**[0146]** The genomic structure of the *SMP1* gene was evaluated by PCR using internal primers and nucleotide sequencing (Fig. 3). The sizes of the *SMP1* introns were estimated by PCR amplicons obtained with primers rend32, sr9, sf1c, sf1, sm19, sr45, sr47, sr47c, sr5, sr5c, sr55, sr55c, sr3, sr3kp, rea7. The positions of the intron/exon junctions and the absence of additional introns were determined by nucleotide sequencing. Six introns could be identified. Exon 1 contained 5' untranslated sequences only and was separated from the Rhesus *box* by 15 bp. The long 3' untranslated sequence of exon 7 overlapped with *RHCE* exon 10. The total gene size was estimated to be 20,000 bp resulting, in conjunction with the downstream *Rhesus box,* in a distance between *RHD* and *RHCE* of about 30,000 bp (Fig. 1).

**Example 10: Localization of the *RHD* gene deletion in the *RHD* negative haplotypes**

**[0147]** It was reasoned that the homology of the two *Rhesus boxes* may have been instrumental for the mechanism of the *RHD* deletion in the common *RHD* negative haplotypes. The nucleotide sequence of the *Rhesus box* in *RHD* negative DNA was determined (Fig. 5). The single *Rhesus box* detected in *RHD* negatives had a hybrid structure. The 5' end of this *Rhesus box* represented a upstream *Rhesus box*, the 3' end a downstream *Rhesus box.* It was determined that the 903 bp breakpoint region of the *RHD* deletion was located in the identity region of the *Rhesus boxes* (Fig. 4, arrow pointing to left).

**Example 11: Specific detection of the *RHD* deletion by PCR**

**[0148]** Two PCR based methods were developed for specific detection of the *RHD* gene deletion occurring in the prevalent *RHD* negative haplotypes (Fig. 6). Long-range PCR-SSP was performed using the expand long template PCR system with buffer 3 and primers rez4 (5' of upstream *Rhesus box)* and sr9 (*SMP1* exon 1). Annealing was at 60°C and extension 20 min at 68°C. PCR amplicons were resolved using a 1% agarose gel. PCR-RFLP was performed using the expand high fidelity PCR system and primers rez7 (non-specific, 5' of *Rhesus box* identity region) and mb31 (specific for downstream *Rhesus box,* 3' of *Rhesus box* identity region). Annealing was at 65°C and extension 10 min at 68°C. PCR amplicons were digested with *Pst*I for 3 hrs at 37°C and fragments resolved using a 1 % agarose gel. These techniques allowed the ready and direct detection of the common *RHD* negative haplotypes, even if they are *in trans* to *RHD* positive haplotypes. PCR-RFLP was further applied to a larger number of samples (Table 3). As expected, all 33 samples with known genotype were correctly typed. In 68 additional samples representative of the most common phenotypes, the results were consistent with the known haplotype frequencies in the population.

**Example 12: *RHD* PCR-SSP**

**[0149]** The PCR-SSP reactions (Table 4) were adapted and extended from a previously described *RHD* exon specific PCR-SSP method (Gassner, Transfusion 37:1020-6, 1997) and were triggered to work under identical thermocycling conditions. Concentrations of specific primers were 0.2 μM for all reactions with the exception of exon 6 (0.1 μM), intron 7 (0.4 μM) and exon 9 (0.4 μM). For most samples intron 4/exon 7 was tested as multiplex reaction containing 0.2 μM of exon 7 (primer set ga71/ga72) and 0.1 μM of intron 4 primers. Each reaction contained a set of HGH primers (Gassner, Transfusion 37:1020-6, 1997) as an internal control in concentrations of 0.05 μM for promoter, intron 4, and exon 7 with ga71/ga72; 0.075μM for exon 10; 0.1 μM for intron 7; 0.15 μM for exon 3, exon 4, exon 7 with rb26/re71, and exon 9; 0.2 μM for exon 5 and exon 6. $Mg^{2+}$ concentration was 0.4 μM for intron 7 and for all other reactions 0.15 μM. For exon 6, 20 % solution Q (Qiagen, Hilden, Germany) was added.

**Example 13. Improved *RHD* PCR-SSP for routine DNA typing.**

**[0150]** Based on the alleles detected in this study and described previously, we devised an improved RHD PCR-SSP for routine DNA typing that included the specific detection of RHDΨ and alleles detected in this study, like RHD(W16X) in a single PCR tube. Reaction A contained primers ga71 and ga72 at 0.3 μM, rb12 and re41 at 0.1 μM, and HGH primers at 0.1 μM. Mg2+ was at 0.175 μM. Reaction B contained primers RhPsiF and RhPsiB at 0.5 μM, re11d and

RhX1f1 at 0.3 μM, re721 and rb9 at 0.2 μM and as control primers rend9b1 and rend 9b2 at 0.2 μM. Primer sequences were ga71, gttgtaaccgagtgctggggattc; ga72, tgccggctccgacggtatc; rb12, tcctgaacctgctctgtgaagtgc;.re41, cgatacccagtttgtctgccatgc; RhPsiF, agacagactaccacatgaacttac; RhPsiB, tctgatctttatcctccgttccctc; re11d, agaagatgggggaatctttttcct; RhX1f1. cgctgcctgcccctctga; re721, ctggaggctctgagaggttgag; rb9, aagctgagttccccaatgctgagg; rend9b1, cactgcacttggcaccattgag; rend9b2, ttccgaaggctgcttttccc.

**[0151]** The PCR reactions could be performed in two tubes (Fig. 15), tested five polymorphisms and were expected to have a false-positive rate of less than 1:10,000 (Table 11).

### Example 14: PCR reactions for Cde$^s$

**[0152]** A hybrid exon 3 with a N152T substitution occurring in the Cde$^s$ haplotype was detected by a PCR-SSP reaction using specific primers Rh152Tb and ga31 at 0.3 μM. The L245V substitution observed in Cde$^s$ was detected with specific primers Rh223Vf and Rh245Vb at 0.2 μM. HGH primer concentrations were 0.1 μM. The other PCR conditions were identical as described in the previous paragraph. Primers sequences were Rh152Tb, gatattactgatgaccatcctcatgg; Rh223Vf, ttgtggatgttctggccaagtg; and Rh245Vb, gctgtcaccactctgactgctac. The *Cde$^s$* haplotype, that is frequent in Africans (Faas, Transfusion 37:38-44, 1997; Singleton, Blood 95:12-8, 2000), possesses a hybrid exon 3 harboring the *RHCE* specific N152T substitution (Faas, Transfusion 37:38-44, 1997). This hybrid exon is expected to be typed as *RHD* positive by the *RHD* exon 3 specific PCR that detected an A at position 383 (codon 128) and was used in the population survey. Since pattern 4 and pattern 8 were compatible with the known data about the *Cde$^s$* haplotype, the presence of a hybrid exon 3 was evaluated in the two samples by sequencing the 3' part of exon 3 and by a PCR-SSP specific for an exon 3 hybrid indicative of *Cde$^s$*. The pattern 4 sample possessed a normal *RHD* exon 3, while the pattern 8 sample had a hybrid exon 3 as predicted for a *Cde$^s$* haplotype. Also, the T at position 410 (A137V substitution) typical for the *Cde$^s$* haplotype (Daniels, Transfusion 38:951-8, 1998) and also present in *D category III type IV* was detected. The identity of pattern 8 and *Cde$^s$* was further corroborated by a PCR-SSP detecting G at position 733 (L245V substitution).

### Example 15: 5' breakpoint region of, *Cde$^s$* in intron 3.

**[0153]** Based on its cDNA, *Cde$^s$* had been characterized as an *RHD-CE(3-7)-D* hybrid gene, in which the 5' part of exon 3 derived from *RHD* and the 3' part of exon 3 including codon 152 derived from *RHCE.* We noted that a similar hybrid exon 3 with a N152T substitution was found in D category III type IV (Wagner, F.F., Frohmajer, A., Ladewig, B., Eicher, N.L, Lonicer, C.B., Müller, T.H., Siegel, M.H., and Flegel, W.A. Weak D alleles express distinct phenotypes. Blood 95:2699-2708, 2000) and in D category IVa (Rouillac, C., Colin, Y., Hughes-Jones, N.C., Beolet, M., D'Ambrosio, A.-M., Cartron, J.P., and Le Van Kim, C. Transcript analysis of D category phenotypes predicts hybrid Rh D-CE-D proteins associated with alteration of D epitopes. Blood 85:2937-2944, 1995), two aberrant *RHD* alleles in which exons 4 to 7 derived from *RHD.* We reasoned that the N152T substitution might have antedated the substitution of *RHD* exons 4 o 7 in *Cde$^s$*. In this case, the 5' breakpoint region was expected to be located in intron 3 rather than exon 3 as predicted from the cDNA. We hence evaluated the presence of *RHD* specific polymorphisms in, *Cde$^s$* intron 3.

**[0154]** To evaluate the presence of the *Eco*RV-site at nucleotide position 752 (*RHD* specific) and 2872 (*RHCE* Specific) and of the *Pvu*II-site at nucleotide position 1777 (*RHCE* specific), the 5' part of intron 3 of *RHD* and *RHCE* was amplified using primers rb3 and rb33 and digested with *Eco*RV or *Pvu*II. To evaluate the presence of the *Sac*I-site at nucleotide position 7797 (*RHCE* specific) and of the *Alw44*I-site at nucleotide position 8550 (*RHD* specific), the 3' part of intron 3 of *RHD* and *RHCE* was amplified using primers rb34 and rb5 and digested with *Sac*I or *Alw44*I. Primer sequences were rb3, aaggtcaacttggcgcagttggtgg; rb33, gtgagactgagttctgtattctgg; rb34, ccagaatacagaactcagtctcac; rb5, ggcagacaaactgggtatcgttgc.

**[0155]** The PCR-RFLP analysis of these intron 3 polymorphisms indicated that *RHD* specific sequences were present at least up to intron 3 position 2872. To further determine the 5' breakpoint region of *Cde$^s$*, we sequenced a DNA stretch encompassing the breakpoint region. DNA was amplified using primers rb3 and re37 and sequenced using primers rb33, rb34 and Cdesf1. Primer sequences were re37, gggttaaagtcacatacacagatg; Cdesf1, atacagaactcagtctcacaacttag. We determined that the breakpoint region was located in intron 3 as shown in Figure 12.

### Example 16: 3' breakpoint region of *Cde$^s$* in intron 7.

**[0156]** To determine the 3' breakpoint region of *Cde$^s$* in intron 7, we sequenced parts of intron 7. DNA was amplified using sense primers rb8, re77 and rex1 and antisense primers rb51 and re711b. Primers rb43, rex19c, cdes7b2, and cdes7f2 were used for nucleotide sequencing. Primer sequences were rb8, gtgttgtaaccgagtgctgggg; re77, tctccacagctccatcatggg; rex1, ggctgtaaaaatggctgaagcag; rb51, gcatgacgtgttctgcctcttg; re711b, ctatcagcattctgatctcaacg; rb43, gaatagcagagaaaaxtcagactgcc; rex19c, gctccattcttgacaatacaggc; cdes7b2, gcttatactatataagttgggtttttgg; cdes7f2, gtttgaatcccaagagccactcat. We established the breakpoint region as shown in Fig. 13. The structure of the 3' breakpoint region

was intriguing, because there were multiple switches between *RHCE* and *RHD* specific sequences. Those features are unusual for a breakpoint region and may be used for specific diagnosis of *$Cde^s$*. They may indicate that the parental alleles differed from the standard *RHCE* and *RHD* sequences or that after the major gene conversion, additional small gene conversions were introduced.

**Example 17: A. PCR-SSP to specifically detect *$Cde^s$*.**

**[0157]** Usually, the presence of *$Cde^s$* is identified by the *RHD-CE-D* hybrid pattern in an *RHD* exon specific PCR. Such an approach does not allow the specific detection of the D negative *$Cde^s$* haplotype, if an *RHD* positive haplotype occurs *in trans*. Since $Cde^s$ does not contain a hybrid *Rhesus box, a RHD*/*$Cde^s$* heterozygous person is likely mistyped as *$RHD^+$*/*$RHD^+$* homozygous. There are several distinct features of *$Cde^s$* in the promoter, intron 2, exon 2, and exon 3 that might be used for a specific detection. These features are, however, shared by the D positive alleles D category IVa and partially by D category III type IV, which would hence confound such methods of detection.

**[0158]** Based on the *$Cde^s$* specific DNA sequence in intron 7, we developed a PCR-SSP that specifically detected *$Cde^s$*. The 3' breakpoint region of *$Cde^s$* in intron 7 was detected by PCR-SSP using specific primers Cdes7f2 and Cdes7b2 at 0.4 $\mu$M and HGH control primers at 0.15 $\mu$M. The other PCR conditions were identical as described in example 12. Primer sequences were Cdes7f2, gtttgaatcccaagagccactcat; Cdes7b2, gcttatactatataagttgggtttttgg. We obtained a specific product with the index *$Cde^s$* sample (Figure 14), two additional *$Cde^s$* samples and a *$RHD\Psi$*/*$Cde^s$* heterozygous sample (Figure 14). Normal *RHD* positive and *RHD* negative samples as well as samples of D category III type IV and of D category IVa did not result in a specific PCR product (Figure 14). We concluded that our PCR-SSP method allowed a specific detection of *$Cde^s$*, even if it occurred *in trans* to another *RHD* positive allele. Furthermore, the detection method was not confounded by D category III type IV or D category IVa that shared the N152T substitution with *$Cde^s$*. It should be noted that the latter haplotypes are frequent in populations comprising African ethnic background, in which *$Cde^s$* is prevalent. The method described by us in this example allowed the specific detection of *$Cde^s$*, is not confounded by the other haplotypes and hence represents a considerable improvement to the prior art. Our characterization of the 5' breakpoint region (example 15) will likewise allow the specific detection of *$Cde^s$* by any suitable method known in the art, like PCR-SSP, PCR-LP, PCR-RFLP, PCR-SSO, Southern blotting etc.

**[0159]** The specific detection of *$Cde^s$* is also important for the correct prediction of the antigen C. The *RHD* gene of *$Cde^s$* encodes for an antigen C that is often missed in DNA based methods for the prediction of antigen C.

**Example 18: Immunohematology**

**[0160]** One sample of each *RHD* positive allele was evaluated by direct agglutination with two monoclonal anti-D (Seraclon anti-D, clone BS226; Biotest, Dreieich, Germany, and Frekaklon anti-D, clone MS201; Gull, Bad Homburg, Germany). Indirect antiglobulin test was done in a gel matrix test (LISS-Coombs 37 °C, DiaMed-ID Micro Typing System, DiaMed. Cressier sur Morat, Switzerland) using an oligoclonal anti-D (Seraclon anti-D blend, clones H41 11B7, BS221 and BS232; Biotest). Samples reactive in gel matrix technique were further investigated using the monoclonal anti-D HM10, HM16, P3x61, P3x35, P3x212 11F1, P3x212 23B10, P3x241, P3x249, P3x290 (Diagast, Loos, France) and H41 11B7 (Biotest). The presence of a $D_{ed}$ phenotype was determined by adsorption of 500 $\mu$l of a polyclonal anti-D (Human incomplete anti-D; Lome Laboratories, Reading, UK) to 500 $\mu$l red cells for 1 h at 37 °C and elution using a chloroform technique (Flegel, Transfusion 40:428-434, 2000). The analysis of samples routinely grouped as D negative revealed 16 $D_{el}$ samples and 3 D positive samples with weak or partial D. These samples clustered among samples previously believed to be D negative with a C or E (Table 9). Nineteen of twenty-seven discrepancies between routine serology and a PCR testing intron 4 and exon 7 represented D positive samples missed by serology, only eight were due to false-positive PCR.

**Example 19: Haplotype frequencies**

**[0161]** For alleles observed more than once, their haplotype association was trivial. Alleles that were observed only once were assumed to be associated with the Cde or cdE. haplotype rather than the cde haplotype, because no *RHD* positive allele was detected in any ccddee sample. An allele occurring in a single CcddEe sample was formally counted half for Cde and half for cdE. CCddee samples were assumed to harbour one aberrant and one normal Cde allele. The frequency of a given aberrant *RHD* allele in its haplotype was calculated as the number of observed samples divided by the number of the corresponding haplotypes under observation (500 Cde, 302 cdE). The population frequency of an *RHD* allele was calculated from the frequency of this allele in its haplotype and the known frequency of the haplotype in the local population (Wagner, Infusionsther. Transfusionsmed. 22:285-90, 1995). The haplotype frequencies were calculated for each PCR pattern and for each *RHD* allele (Table 8). In accordance with a previous study in England by Avent et al. (Avent, Blood 89:2568-77,1997), 4.9% of Cde haplotypes and 1.5% of cdE haplotypes were *RHD* positive

in our population. As no *RHD* positive allele was detected among 314 ccddee samples, the frequency in the cde haplotype was less than 0.5 % (upper limit of one-sided 95% confidence interval, Poisson distribution). The three frequencies differed statistically significantly from each other ($p<0.05$; two sided Fisher's exact test for each pairwise comparison corrected according to Bonferoni-Holm). The population frequency of any D negative *RHD* positive haplotype was estimated to be 1:1,606. $D_{el}$ alleles could only be observed in the presumed Cde haplotypes. About 3% of samples carrying antigen C that were typed D-negative in the blood bank routine represented $D_{el}$. The population frequency of $D_{el}$ alleles was 1:3,030.

**Table 1. Primers**

| Primer | Nucleotide sequence | Localization | Position |
|---|---|---|---|
| rb10b | ggctaaatattttgatgaccaagtt | *RHD* cDNA | 1,194 to 1,217 |
| re011d | gcagccaacttcccctgtg | *RHD* promoter | -883 to -901 |
| re014 | gctctaccttggtcacctcc | dJ469D22 | 52,189 to 52,209 |
| reD4 | aggtcacatccatttatcccactg | dJ469D22 | 53, 968 to 53,945 |
| re11d | agaagatgggggaatctttttcct | dJ469D22 | 51,193 to 51,216 |
| re96 | ttgtgactgggctagaaagaaggtg | dJ469D22 | 242 to 216 |
| rea7 | tgttgcctgcatttgtacgtgag | *RHD* cDNA | 1,311 to 1,333 |
| rend31 | ttctgtctgggttggggaggg | dJ465N24 | 128,649 tn 128,629 |
| rend32 | ggaggggttaatatgggtggc | dJ465N24 | 127,355 to 127,375 |
| rend8b1 | tttgtcctggttgcctgtggtc | dJ465N24 | 69,296 to 69.274 |
| rend8b2 | caaatcctgttgactggtctcgg | dJ465N24 | 68.451 to 68,473 |
| rend9a1 | aacggctccatcacccctaaag | dJ465N24 | 50,008 to 49,987 |
| rend9a2 | cccactcctagataccaacccaag | dJ465N24 | 49,059 to 49,083 |
| rey14a | ctttatgcactgcctcgttgaatc | dJ469D22 | 56,792 to 56,769 |
| rey14b | ttgactggtgtggttgctgttg | dJ469D22 | 55,863 to 55,884 |
| rey15a | gcagaaaggggagttgatgctg | dJ469D22 | 55,416 to 55,395 |
| rey7 | ctgacaaagttgagagcccactg | dJ469D22 | 62,324 to 62,346 |
| rey8 | ttaagcctacatccacatgctgag | dJ469D22 | 62,854 to 62,831 |
| rez2 | ccttggtctgccagaattttca | *RHD* cDNA | 2738 to 2717 |
| rez4 | gtttggcatcataggagatttggc | dJ465N24 | 120,101 to 120,124 |
| rez7 | cctgtccccatgattcagttacc | dJ465N24 | 124,831 to 124,854 |
| rh7 | acgtacaaatgcaggcaac | *RHD* cDNA | 1,330 to 1,312 |
| rnb31 | cctttttttgtttgtttttggcggtgc | downstream *Rhesus box* | 6,710 to 6,684 |
| rr4 | agcttactggatgaccacca | *RHD* cDNA | 1,541 to 1,522 |
| sf1 | gactggggggaaaagcgcaatac | *SMP1* cDNA | 142 to 164 |
| sf1c | gtattgcgcttttccccccagtc | *SMP1* cDNA | 164 to 142 |
| sf3 | tgacttgctctcatcccacatg | *SMP1* cDNA | 1,696 to 1,717 |
| sm19 | gggcttgaagcaagtaaatggaag | *SMP1* intron 1 | .58 to -35 |
| sr1 | gctatcaatattttcttggttacagacac | *SMP1* cDNA | 2,172 to 2,144 |
| sr3 | gttcactgccataagtcttcagtgc | *SMP1* cDNA | 575 to 551 |
| sr3kp | tggccgcactgaagacttatgg | *SMP1* cDNA | 546 to 567 |
| sr45 | cagctgcatctatgataatccacc | *SMP1* cDNA | 224 to243 |
| sr47 | atggacaagtccgaggtgatag | *SMP1* cDNA | 315 to 344 |
| sr47c | atcacctcggacttgtccattc | *SMP1* cDNA | 342 to 321 |
| sr5 | gcaatcagagatccaaaggccaac | *SMP1* cDNA | 428 to 405 |
| sr5c | gttggcctttggatctctgattgc | *SMP1* cDNA | 405 to 428 |
| sr55 | gacatagtataccctggaattgctgt | *SMP1* cDNA | 472 to 497 |
| sr55c | acagcaattccagggtatactatgtc | *SMP1* cDNA | 497 to 472 |

(continued)

| Primer | Nucleotide sequence | Localization | Position |
|---|---|---|---|
| sr9 | ctcccccgattttagccaagaa | *SMP1* cDNA | 27 to 6 |

For the *RHD* promoter and the *RHD* cDNA, the positions refer to the distance from the A of the start codon. For introns, they refer to the distance from the intron/exon junction. For all other sequences including the *SMP1* cDNA, they refer to the distance from the start of the published sequences. The mismatches in primers rey14b, rnb31, and sf3 were inadvertently introduced. Primers re11d, re014 and re04 do not exactly match dJ469D22, because they were designed from our raw sequences covering the 5' flanking region of *RHD.*

**Table 2**. Presence of *RHD* flanking sequences in the YAC 38A-A10

| Primer | | | | Amplicons obtained with | | |
|---|---|---|---|---|---|---|
| | | | Amplicon | Genomic DNA | | YAC |
| **sense** | **antisense** | **Predicted position** | **size** | **RHD⁺** | **RHD⁻** | **38A-A10** |
| **rend9a1** | **rend9a2** | ***RHD* 5' flanking region about 85,000 bp from ATG** | **948 bp** | **yes** | **yes** | **yes** |
| **rend8b1** | **rend8b2** | ***RHD* 5' flanking region about 50,000 bp from ATG** | **845 bp** | **yes** | **yes** | **yes** |
| **rea7.** | **rez2** | ***RHD* 3' flanking region about 1,500 bp from STOP** | **1,412 bp** | **yes** | **no** | **yes** |
| **rend32** | **sr9** | ***RHCE* 3' flanking region about 20,000 bp from STOP** | **1,989 bp** | **yes** | **yes** | **yes** |
| **sr1** | **sf3** | ***RHCE* 3' flanking region about 1,000 bp from STOP** | **477 bp** | **yes** | **yes** | **yes** |
| **rey14b** | **rey14a** | ***RHCE* 5' flanking region about 5,300 bp from ATG** | **929 bp** | **yes** | **yes** | **no** |
| **rey7** | **rey8** | ***RHCE* 5' flanking region about 10,000 bp from ATG** | **530 bp** | **yes** | **yes** | **no** |

**Table 3.** PCR-RFLP for the specific detection of the *RHD* deletion.

| | | | Number of samples with *RHD* genotype | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Known | Samples | determined | | | expected | | | |
| Phenotype | genotype | tested (n) | +/+ | +/- | -/- | +/+ | +/- | -/- | *P*¶ |
| *Known genotype* | | | | | | | | | |
| ccddee | cde/cde | 14 | 0 | 0 | 14 | 0 | 0 | 14 | N.A. |
| CCddee | Cde/Cde† | 5 | 0 | 0 | 5 | 0 | 0 | 5 | N.A. |
| ccddEE | cdE/cdE† | 1 | 0 | 0 | 1 | 0 | 0 | 1 | N.A. |
| D variants | D/cde‡ | 9 | 0 | 9 | 0 | 0 | 9 | 0 | N.A. |
| ccDEe | cDe/cDE§ | 4 | 4 | 0 | 0 | 4 | 0 | 0 | N.A. |
| *Common phenotypes* | | | | | | | | | |
| CcDee | | 10 | 1 | 9 | 0 | 0.5 | 9.5 | 0 | >0.4 |

(continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Common phenotypes* | | | | | | | | |
| ccDEe | 10 | 0 | 10 | 0 | 0.3 | 9.7 | 0 | >0.5 |
| ccDee | 10 | 1 | 9 | 0 | 0.5 | 9.5 | 0 | >0.4 |
| CCDee | 10 | 9 | 1 | 0 | 9.5 | 0.5 | 0 | >0.4 |
| CcDEe | 12 | 11 | 1 | 0 | 11 | 1 | 0 | >0.5 |
| ccDEE | 10 | 10 | 0 | 0 | 9.2 | 0.8 | 0 | >0.4 |
| CCDEe | 6 | 5 | 1 | 0 | 5.8 | 0.2 | 0 | >0.1 |

* Expected number of $RHD^+/RHD^+$ and $RHD^+/RHD^-$ samples based on known genotypes or the haplotype frequencies in the local population [41]

† *RHD*-negative in PCR.

‡ $RHD^+/RHD^-$, because a weak or partial D phenotype would be masked in a $RHD^+/RHD^+$ gentoype. These samples were weak D type 1 (n=2), type 2 (n=2), type 3 (n=2), type 4 (n=2) and $D^{VII}$ (n=1).

§ Presence of two *RHD* genes differing in their polymorphic *Hae*III-site in intron 3 [42] demonstrated by PCR-RFLP.

N.A. - not applicable. Probabilities were calculated based on confidence limits of binomial distribution.

**Table 4. *RHD* PCR-SSP**

| Region | Name Reference | DNA sequence | Position | | Polymorphisms detected | Amplicon size | |
|---|---|---|---|---|---|---|---|
| Promoter | re012 | tccactttccacctccctgc | Promoter | -1,137 to -1,1197 bp deletion at -1125 | | 255 | 43 |
| | re011d | gcagccaacttcccctgtg | Promoter | -883 to -901 4 bp deletion at -896 | | | 44 |
| Exon 3 | ga31 (D-3-383) | ttgtcggtgctgatctcagtgga | Exon 3 | 361 to 383 | 383 A | 154 | 21 |
| | rb21 | aggtccctcctccagcac | Intron 3 | 28 to 11 | | | 42 |
| Exon 4 | ga41 (D-4-527) | acatgatgcacatctacgtgttcgc | Exon 4 | 503 to 527 | | 123 | 21 |
| | ga42 (D-4-602) | cagacaaactgggtatcgttgctg | Exon 4 | 625 to 602 | 602 C | | 21 |
| Intron 4 | re41 this study | cgatacccagtttgtctgccatgc | Exon 4 | 608 to 631 | | 226 | |
| | rb12 | tcctgaacctgctctgtgaagtgc | Intron 4 | 198 to 175 | Intron 4 deletion in *RHD* | | 40 |
| Exon 5 | rb24 | agacctttggagcaggagtg | Intron 4 | -53 to -34 | | 228 | 40 |
| | ga51 (D-5-787) | ctgctcaccttgctgatcttccc | Intron 5/Exon5 | | 8 to 787 | 787 G | 21 |
| Exon 6 | ga62 (D-6-826) | ttatgtgcacagtgcggtgttgg | Exon 6 | 804 to 826 | | 133 | 21 |
| | ga61 (D-6-916) | caggtacttggctcccccgac | Exon 6 | 936 to 916 | 916G | | 21 |
| Exon 7† | ga71 (D-7-967) | gttgtaaccgagtgctggggattc | Exon 7 | 944 to 967 | | 123 | 21 |
| | ga72 (D-7-1048) | tgccggctccgacggtatc | Exon 7 | 1,066 to 1,048 | 1,048 G | | 21 |

(continued)

| Region | Name Reference | DNA sequence | Position | | Polymorphisms detected | Amplicon size | |
|---|---|---|---|---|---|---|---|
| Exon 7† | rb26 | aggggtgggtagggaatatg | Intron 6 | -62 to -43 | | 130 | 42 |
| | re71 | acccagcaagctgaagttgtagcc | Exon 7 | 1,008 to 985 | 985/986 GG | | 42 |
| Intron 7 | rb52 | ccaggttgttaagcattgctgtacc | Intron 7 | 6,666 to 6,690 | 6,690 C | | 42 |
| | rb51 | gcatgacgtgttctgcctcttg | Intron 7 | 6,734 to 6,713 | 6,713 C | 169 | |
| | this study | | | | | | |
| Exon 9 | re83 | gagattaaaaatcctgtgctcca | Intron 8 | -56 to -34 | | 119 | 42 |
| | re94 | cttggtcatcaaaatatttagcct | Exon 9 | 1,216 to 1,193 | 1,193 A | | |
| | this study | | | | | | |
| Exon 10 (3' UTR) | rea7 | tgttgcctgcatttgtacgtgag | 3'UTR | 1,310 to 1,333 | *RHD*/*Rhesus box* | *23* | 44 |
| | rr4 | agcttactggatgaccacca | 3'UTR | 1,541 to 1,522 | junction | | 42 |

* Primer names in brackets are as described by Gassner et al. [21].
† Primer set ga71/ga72 was used for the screening, primer set rb26-re71 for *RHD* exon specific PCR-SSP.

**Table 5.** Population survey of known D negative blood donors screened by *RHD* PCR-SSP

| Documented phenotype | Samples (n) screened | Samples (n) PCR-SSP positive* |
|---|---|---|
| ccddee | 314 | 0 |
| Ccddee | 433 | 34 |
| ccddEe | 271 | 5 |
| CCddee | 24 | 4 |
| CcddEe | 19 | 4 |
| ccddEE | 6 | 1 |
| CcddEE | 1 | 0 |
| Total | 1,068 | 48 |

Positive for at least one of four *RHD* specific polymorphisms tested (promoter, intron 4, exon 7 or 3' UTR).

**Table 6.** PCR patterns compatible with *RHD-RHCE-RHD* hybrid genes or partial *RHD* deletions in 25 D negative samples

| PCR | *RHD* specific PCR-SSP* | Samples | Phenotype | | | | |
|---|---|---|---|---|---|---|---|
| pattern | P E3E414 E5E6E717 E9E10 | Possible cause† | (n) | Documented | Confirmed | Haplotype | Reference‡ |
| Pattern 1 | + - - - - - - - - + | *RHD-CE (3-9)-D* | 11 | Ccddee§ | D negative | Cde | Whites [1,25] |
| Africans [24] | | | | | | | |
| Pattern 2 | + - - - - - - - + + | *RHD-CE (3-7)-D* | 4 | Ccddee | D negative | Cde | this study |
| Pattern 3 | + + - - - - - - + + | *RHD-CE (4-7)-D* | 3 | ccddEe | D negative | cdE | Whites [16] |
| Pattern 4 | + + - - - - - + + + | *RHD-CE (4-7)-D* | 1 | CcddEe | D negative | n.k.¶ | this study |
| Pattern 5 | + + - - - + + + + + | *RHD-CE (4-5)-D* | 2 | ccddEe$^{\alpha}$ | partial D/$D_{el}$$^{\alpha}$ | cDE | Whites [3,22,30,40] |
| Pattern 6 | + + + + + + + - - + | *RHD-CE (8-9)-D* | 3 | CCddee | D negative | Cde | Whites [21] |
| Pattern 7 | - - - - - - - - - + | *RHCE (1-9)-D (10)* | 1 | ccddEe | D negative | cdE | this study |
| Pattern 8 | - + - - - - - + + + | *RHD (1-3)-CE (4-7)-D* | 1 | CcddEe | D negative | Cde$^{\beta}$ | Africans [5,15] |

* P - Promoter, E3 - Exon 3, E4 - Exon 4,14 - Intron 4, E5 - Exon 5, E6 - Exon 6; E7 - Exon 7; 17 - Intron 7; E9 - Exon 9; E10 - Exon 10 (3' UTR)
† Assuming the presence of a single *RHD-CE-D* hybrid allele.
‡ Previously described alleles that fit PCR pattern and haplotype.
§ 11 samples: 9 Ccddee, 1 CCddee, 1 CcddEe
¶ n.k. - not known.
$^{\alpha}$ 2 samples, 1 labeled CcddEe with $D_{el}$ phenotype, 1 labeled ccddEe with partial D $D^{VI}$ phenotype.
$^{\beta}$ Probably identical to Cde$^{s}$ (see below).

**Table 7.** *RHD* alleles with single nucleotide substitutions in 22 D negative samples

| | | | Samples | Phenotype | | | |
|---|---|---|---|---|---|---|---|
| *Allele* | Substitution Reference | Effect(s) | (n) | Documented | Confirmed | Haplotype | |
| *RHD*(W16X) study | G->A at 48 | Stop codon at codon 16 | 2 | Ccddee | D negative | Cde | this |
| *RHD*(G486 (+1)A) study | g->a at 486+1 | 5' splice site Intron 3 ACgt->ACat | 3 | Ccddee | $D_{el}$ | CDe | this |
| *RHD*(G212V) study | G->T at 635 | 3' splice site intron 4 agGC->agTC Missense mutation G212V | 1 | Ccddee | D negative | Cde | this |

(continued)

| Allele | Substitution Reference | Effect(s) | Samples (n) | Phenotype Documented | Phenotype Confirmed | Haplotype | |
|---|---|---|---|---|---|---|---|
| *RHD*(C285Y) study[1] | G->A at 854 | Missense mutation C285Y | 1 | ccddEe | partial D* | cDE | this |
| *RHD*(M2951) | G->T at 885 | Missense mutation M2951 | 7 | Ccddee | $D_{el}$ | CDe† | 42 |
| *RHD*(Y330X) study | C->G at 985 | Stop codon at codon 330 | 1 | Ccddee | D negative | Cde | this |
| *RHD*(G1153 (+1)A) study | g->a at 1153+1 | 5'splice site intron 8 AGgt->AGat | 1 | Ccddee | D negative | Cde | this |
| *RHD*(G385A) | G->C at 1154 | 3' splice site intron 8 agGT->agCT Missense mutation G385A | 1 | CcddEe | weak D | cDE | 42 |
| *RHD*(K409K) study | G->A at 1227 | 5' splice site intron 9 AGgt->AAgt | 5 | Ccddee | $D_{el}$ | CDe | this |

* A detailed serologic analysis of this sample representing the partial D DIM has been published previously [43].
† The same allele occurring in a cDe haplotype has been described as weak D type 11.

**Table 8.** Estimated frequencies in population

| PCR pattern/Allele | Frequency among Cde/ cdE | Frequency in population |
|---|---|---|
| Pattern 1 | 1:45 | 1:4,132 |
| Pattern 2 | 1:125 | 1:11,364 |
| Pattern 3 | 1:101 | 1:17,976 |
| Pattern 4 | 1:500* | 1:45,455* |
| Pattern 6 | 1:167 | 1:15,152 |
| Pattern 7 | 1:302 | 1:53,929 |
| Pattern 8 | 1:500 | 1:45,455 |
| *RHD*(W16X) | 1:250 | 1:22,727 |
| RHD(G212V) | 1:500 | 1:45,455 |
| *RHD*(Y330X) | 1:500 | 1:45,455 |
| *RHD*(G1153(+1)A) | 1:500 | 1:45,455 |
| Any D negative | 1:20 / 1:67† | 1:1,607 |
| *RHD*(G486(+1)A) | 1:167 | 1:15,152 |
| *RHD*(M295I) | 1:71 | 1:6,493 |
| *RHD*(K409K) | 1:100 | 1:9,091 |

(continued)

| PCR pattern/Allele | Frequency among Cde/ cdE | Frequency in population |
|---|---|---|
| Any $D_{el}$ | 1:33‡ | 1:3,030 |

* Assuming a Cde haplotype; a cdE haplotype would result in a frequency of 1:302 among cdE and 1:53,929 in the population. For statistics and sum frequencies, the haplotype was formally counted as 0.5 Cde and 0.5 cdE.
† 1:20 among Cde, 1:67 among cdE.
‡ 1:33 relative to the Cde haplotype.

**Table 9** False negative rate in routine typing for antigen D

| Documented Phenotype | Samples (n) | Confirmed phenotype (n) $D_{el}$ | Confirmed phenotype (n) partial or weak D | False negatives n | False negatives Rate |
|---|---|---|---|---|---|
| ccddee | 314 | 0 | 0 | 0 | 0%† |
| Ccddee | 433 | 15 | 0 | 15 | 3.5% |
| ccddEe | 271 | 0 | 2 | 2 | 0.7% |
| other' | 50 | 1 | 1 | 2 | 4% |
| D negative | N.A. ‡ | 0.15% | 0.02% | | 0.17% |

* CCddee, CcddEe, ccddEE, and CCddEe
† Upper limit of 95% confidence interval was 0.95% (Poisson distribution)
‡ N.A. - not applicable. The frequencies are estimates based on the phenotype frequencies in the population [41].

**Table 10** Previously described D negative, *RHD positive* alleles

| *Allele* | Haplotype | Population | Possible match |
|---|---|---|---|
| *RHD*(Q41X) [2] | Cde | Whites | not detected |
| *RHD-CE(2-9)-D* [1,24,25] | Cde | Whites[1,25], Blacks[24] | Pattern 1 |
| *RHD-CE(3:455-7)-D* [5,15] | Cde[8] | Blacks | Pattern 8 |
| *RHD(*488del4) [1] | Cde | Whites | not detected |
| *RHD-CE(4-7)-D* [16] | cdE | Whites | Pattern 3 or 4 |
| *RHD*Ψ [38] | cde | Blacks | not detected |
| *RHD*(600del) [10] | Cde | somatic mutation* | not detected |
| *RHD* (exon 5 variant) [8] | cde | not communicated | not detected |
| *RHD*(G314V) [34] | Cde | Japanese | not detected |
| *RHD*(exon 9 variant) | Cde | Whites | Pattern 6 |

**Table 11** Population rates of false positives and positive predictive value of different RHD PCR strategies

| PCR strategy | Rate of false positives | Positive predictive value of positive result | Number of polymorphism tested |
|---|---|---|---|
| Exon 10 only | 1:1,275 | 0.999216 | 1 |
| Intron 4/Exon 7 | 1 :4,081 | 0.999755 | 2 |
| Intron 4/Exon 7/*RHD*Ψ | 1:4,700 | 0.999787 | 3 |
| Intron 4/Exon 7/W16X | 1:5,212 | 0.999808 | 3 |
| Intron 4/Exon 7/Intron 7 | 1:6,051 | 0.999835 | 3 |
| Exons 3, 4, 5, 6, 7, 9 | 1:6,051 | 0.999835 | 6 |
| Exons 2, 3, 4, 5, 6, 7, 9, 10 | 1:6,051 | 0.999835 | 8 |

(continued)

**Table 11** Population rates of false positives and positive predictive value of different RHD PCR strategies

| PCR strategy | Rate of false positives | Positive predictive value of positive result | Number of polymorphism tested |
|---|---|---|---|
| Intron 4/Exon 7/W16X/ *RHDΨ* | 1:6,267 | 0.999840 | 4 |
| All Exons/*RHDΨ* | 1:7,520 | 0.999867 | 9 |
| Intron 4/Exon 7/Intron 7/W16X | 1:8,921 | 0.999888 | 4 |
| Intron 4/Exon 7/Intron 7/W16X/*RHDΨ* | 1:12,533 | 0.999920 | 5 |

* Allele acquired by somatic mutation in a woman with chronic myelogenic leucemia and restricted to the myeloid lineage

**References**

**[0162]**


1. Andrews KT, Wolter LC, Saul A, Hyland CA: The RhD- trait in a white patient with the RhCCee phenotype attributed to a four-nucleotide deletion in the RHD gene. Blood 92;1839, 1998

2. Avent ND, Martin PG, Armstrong-Fisher SS, Liu W, Finning KM, Maddocks D, Urbaniak SJ: Evidence of genetic diversity underlying Rh D negative, weak D (Du) and partial D phenotypes as determined by multiplex PCR analysis of the RHD gene. Blood 89:2568, 1997

3. Avent ND, Liu W, Jones JW, Scott ML, Voak D, Pisacka M, Watt J, Fletcher A. Molecular analysis of Rh transcripts and polypeptides from individuals expressing the DVI variant phenotype: an RHD gene deletion event does not generate all DVIccEe phenotypes. Blood 1997;89:1779-86.

4. Blumenfeld OO, Huang CH: Molecular genetics of the glycophorin gene family, the antigens for MNSs blood groups: multiple gene rearrangements and modulation of splice site usage result in extensive diversification. Hum Mutat 6:199, 1995

5. Blunt T, Daniels G, Carritt B: Serotype switching in a partially deleted RHD gene. Vox Sang 67:397, 1994

6. Bowman J: The management of hemolytic disease in the fetus and newborn. Semin Perinatol 21:39,1997

7. Carritt B, Kemp TJ, Poulter M: Evolution of the human RH (rhesus) blood group genes: a 50 year old prediction (partially) fulfilled. Hum Mol Genet 6:843, 1997

8. Carritt B, Steers FJ, Avent ND. Prenatal determination of fetal RhD type. Lancet 1994;344:205-6.

9. Cherif-Zahar B, Mattei MG, Le Van Kim C, Bailly P, Cartron JP, Colin Y: Localization of the human Rh blood group gene structure to chromosome region 1p34.3-1p36.1 by in situ hybridization. Hum Genet 86:398, 1991

10. Cherif-Zahar B, Bony V, Steffensen R, Gane P, Raynal V, Goosens D, Laursen JS, Varming K, Jersild C, Cartron JP. Shift from Rh-positive to Rh-negative phenotype caused by a somatic mutation within the RHD gene in a patient with chronic myelocytic leukaemia. Br J Haernatol 1998;102:1263-70.

11. Colin Y, Cherif-Zahar B, Le Van Kim C, Raynal V, van Huffel V, Cartron J-P. Genetic basis of the RhD-positive and RhD-negative blood group polymorphism as determined by southern analysis. Blood 1991;78:2747-52.

12. Cossu G, Angius A, Gelfi C, Righetti PG: Rh D/d genotyping by quantitative polymerase chain reaction and capillary zone electrophoresis. Electrophoresis 17:1911, 1996

13. Daniels GL, Faas BH, Green CA, Smart E, Maaskant-van Wijk PA, Avent ND, Zondervan HA, von dem Borne AE, van der Schoot CE. The VS and V blood group polymorphisms in Africans: a serologic and molecular analysis. Transfusion 1998;38:951-8.

14. Döscher A, Schunter F, Müller TH: Quantitativae PCR to assess RHD zygosity. Infusionsther Transfusionsmed 26(suppl 1):31, 1999 (abstr.)

15. Faas BHW, Becker EAM, Wildoer P, Ligthart PC, Overbeeke MAM, Zondervan HA, von dem Borne AEGK, van der Schoot CE: Molecular background of VS and weak C expression in blacks. Transfusion 37:38, 1997

16. Faas BHW, Beckers EAM, Simsek S, Overbeeke MAM, Pepper R, van Rhenen DJ, von dem Borne AEGK, van der Schoot CE: Involvement of Ser103 of the Rh polypeptides in G epitope formation. Transfusion 36:506, 1996

17. Filbey D, Hanson U, Wesstrom G: The prevalence of red cell antibodies in pregnancy correlated to the outcome of the newborn: a 12 year study in central Sweden. Acta Obstet Gynecol Scand 74:687, 1995

18. Flegel WA, Khull SR, Wagner FF. Primary anti-D immunization by weak D type 2 RBCs. Transfusion 2000; 40: 428 - 434

19. Flegel WA, Wagner FF, Müller TH, Gassner C: Rh phenotype prediction by DNA typing and its application to practice. Transfus Med 8:281, 1998

20. Fujiwara H, Okuda H, Omi T, Iwarnoto S, Tanaka Y, Takahashi J, Tani Y, Minakami H, Araki S, Sato I, Kajii E: The STR polymorphisms in intron 8 may provide information about the molecular evolution of RH haplotypes. Hum Genet 104:301, 1999

21. Gassner C, Schmarda A, Kilga-Nogler S, Jenny-Feldkircher B, Rainer E, Müller TH, Wagner FF, Flegel WA, Schonitzer D: RhesusD/CE typing by polymerase chain reaction using sequence-specific primers. Transfusion 37: 1020, 1997

22. Huang CH. Human DVI category erythrocytes: correlation of the phenotype with a novel hybrid RhD-CE-D gene but not an internally deleted RhD gene. Blood 1997;89:1834-9.

23. Huang CH, Reid ME, Chen Y, Coghlan G, Okubo Y: Molecular definition of red cell Rh haplotypes by tightly linked SphI RFLPs. Am J Hum Genet 58:133, 1996

24. Huang CH: Alteration of RH gene structure and expression in human dCCee and DCW- red blood cells: phenotypic homozygosity versus genotypic heterozygosity. Blood 88:2326, 1996

25. Hyland CA, Wolter LC, Saul A. Three unrelated Rh D gene polymorphisms identified among blood donors with Rhesus CCee (r'r') phenotypes. Blood 1994;84:321-4.

26. Kemp TJ, Poulter M, Carritt B: Microsatellite variation within the human RHCE gene. Vox Sang 77:159, 1999

27. Lo Y-MD, Bowell PJ, Selinger M, Mackenzie IZ, Chamberlain P, Gillmer MDG, Littlewood TJ, Fleming KA, Wainscoat JS: Prenatal determination of fetal RhD status by analysis of peripheral blood of rhesus negative mothers. Lancet 341:1147, 1993

28. Maaskant-van Wijk PA, Hemker MB, Douglas-Berger L, et al. Ethnic variability of the Rhesus system. Transfusion 1999;39:(10S)103S-4S (Abstract).

29. Maaskant-van Wijk PA, Faas BHW, de Ruijter JAM, Overbeeke MAM, von dem Borne AEGK, van Rhenen DJ, van der Schoot CE. Genotyping of RHD by multiplex polymerase chain reaction analysis of six RHD-specific exons. Transfusion 1998;38:1015-21.

30. Maaskant-van Wijk PA, Beckers EAM, van Rhenen DJ, Mouro I, Colin Y, Carton J-P, Faas BHW, van der Schoot CE, Apoil PA, Blancher A, et al. Evidence that the RHD(VI) deletion genotype does not exist. Blood 1997;90:1709-11.

31. MacGeoch C, Mitchell CJ, Carritt B, Avent ND, Ridgwell K, Tanner MJ, Spurr NK: Assignment of the chromosomal locus of the human 30-kDal Rh (rhesus) blood group-antigen-related protein (Rh30A) to chromosome region 1p36,13-p34. Cytogenet Cell Genet 59:261, 1992

32. Mourant AE, Kopec AC, Domaniewska-Sobczak K: The distribution of the human blood groups and other polymorphisms. London, Oxford University Press, 1976

33. Okuda H, Suganuma H, Tsudo N, Omi T, Iwamoto S, Kajii E: Sequence analysis of the spacer region between the RHD and RHCE genes. Biochem Biophys Res Commun 263:378, 1999

34. Okuda H, Kawano M, Iwamoto S, Tanaka M, Seno T, Okubo Y, Kajii E: The RHD gene is highly detectable in RhD-negative Japanese donors. J Clin Invest 100:373, 1997

35. Onda M, Fukuda M: Detailed physical mapping of the genes encoding glycophorins A, B and E, as revealed by P1 plasmids containing human genomic DNA. Gene 159:225, 1995

36. Reboul J, Gardiner K, Monneron D, Uze G, Lutfalla G: Comparative genomic analysis of the interferon/interleukin-10 receptor gene cluster. Genome Res 9:242, 1999

37. Salvignol I, Calvas P, Socha WW, Colin Y, Le Van Kim C, Bailly P, Ruffie J, Cartron JP, Blancher A: Structural analysis of the RH-like blood group gene products in nonhuman primates. Immunogenetics 41:271, 1995

38. Singleton BK, Green CA, Avent ND, Martin PG, Smart E, Daka A, Narter-Olaga EG, Hawthorne LM, Daniels G: The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in africans with the Rh D- negative blood group phenotype. Blood 95:12, 2000

39. Urbaniak SJ, Robertson AE: A successful program of immunizing Rh-negative male volunteers for anti-D production using frozen/thawed blood. Transfusion 21:64, 1981

40. Wagner FF, Gassner C, Müller TH, Schönitzer D, Schunter F, Flegel WA: Three molecular structures cause Rhesus D category VI phenotypes with distinct immunohematologic features. Blood 91:2157, 1998

41. Wagner FF, Kasulke D, Kerowgan M, Flegel WA: Frequencies of the blood groups ABO, Rhesus, D category VI. Kell, and of clinically relevant high-frequency antigens in South-Westem Germany. Infusionsther Transfusionsmed 22:285,1995

42. Wagner FF, Gassner C, Muller TH, Schonitzer D, Schunter F, Flegel WA: Molecular basis of weak D phenotypes. Blood 93:385, 1999

43. Wagner FF, Frohmajer A, Ladewig B, Eicher Nt, Lonicer CB, Müller TH, Siegel MH, Flegel WA. Weak D alleles express distinct phenotypes. Blood 2000; 95: 2699-2708

44. Wagner FF, Flegel WA. RHD gene deletion occurred in the Rhesus box. Blood 2000;

45. Wissenschaftlicher Beirat der Bundesärztekammer; Paul-Ehrlich-Institut. Richtlinien zur Blutgruppenbestimmung und Bluttransfusion (Hämotherapie). Köln: Deutscher Ärzte-Verlag; 1996.

46. Legler TJ, Kohler M, Mayr WR, Panzer S, Ohto H, Fischer GF; Genotyping of the human platelet antigen systems 1 through 5 by multiplex polymerase chain reaction and ligation-based typing. Transfusion 1996 May; 36(5): 426-31

SEQUENCE LISTING

**[0163]**

<110> DRK Blutspendedienst Baden-Wuerttemberg gGmbH

<120> Molecular structure of RHD negative

<130> D2600 EP/1

<141> 2000-10-31

5 <160> 66

<170> PatentIn Ver. 2.1

<210> 1
10 <211> 100
<212> DNA
<213> Homo sapiens

<400> 1
15

gcatgcgcga ctgagccggg tggatggtac tgctgcatcc gggtgtctgg aggctgtggc 60
cgttttgttt tcttggctaa aatcggggga gtgaggcggg 100

20 <210> 2
<211> 26
<212> DNA
<213> Homo sapiens

25 <400> 2
actgcacgac ggggctggac tgacgt 26

<210> 3
<211> 45
30 <212> DNA
<213> Homo sapiens

<400> 3
agctgaaaaa aatgtctgga tttctagagg gcttgagatg ctcag 45
35

<210> 4
<211> 26
<212> DNA
<213> Homo sapiens
40

<400> 4
gcttccattg ctgctggtgt actagt 26

<210> 5
45 <211> 44
<212> DNA
<213> Homo sapiens

<400> 5
50 agttttttac aggctggtgg attatcatag atgcagctgt tatt 44

<210> 6
<211> 26
<212> DNA
55 <213> Homo sapiens

<400> 6
tatagcaacc atagccttcc taatgt 26

<210> 7
<211> 44
<212> DNA
<213> Homo sapiens

5

<400> 7
aggattaatg cagtatcgaa tggacaagtc cgaggtgata gtta 44

<210> 8
10 <211> 26
<212> DNA
<213> Homo sapiens

<400> 8
15 tgaaggttgt ctgggtcaaa acaggt 26

<210> 9
<211> 44
<212> DNA
20 <213> Homo sapiens

<400> 9
aggtgctcgc atttggcttt tcgttggttt catgttggcc tttg 44

25 <210> 10
<211> 26
<212> DNA
<213> Homo sapiens

30 <400> 10
tttttggagg ttatgttgct aaaggt 26

<210> 11
<211> 38
35 <212> DNA
<213> Homo sapiens

<400> 11
agaaaaagac atagtatacc ctggaattgc tgtatttt 38

40

<210> 12
<211> 26
<212> DNA
<213> Homo sapiens

45

<400> 12
tccagaatgc cttcatcttt tttggt 26

<210> 13
50 <211> 53
<212> DNA
<213> Homo sapiens

<400> 13
55 aggagggctg gtttttaagt ttggccgcac tgaagactta tggcagtgaa cac 53

<210> 14
<211> 249

<212> DNA
<213> Homo sapiens

<400> 14

```
agcatcatcc taatgaaact aaacatttat tttaaactta ttaaattgac tcttaaacta 60
agtttttagt ctttaatttt ttaatatcaa atctgtctct gaccttgttt cattatacat 120
aaggagcttt gctgtcatga gcgtttctca cgtacaaatg caggcaacag tgagaggaag 180
ttgtcttgtt tttgaacagg ccttgttttt cttggatgct tttgcttaaa atccaacagc 240
caaatgagg                                                          249
```

<210> 15
<211> 3093
<212> DNA
<213> Homo sapiens

<400> 15

```
attcactatc acaagaacag cacgggtaag acctgtcccc atgattcagt tacctcccac 60
tgggtccctc ccacaacgca tgggaattca ggatgagatt tgggtgggga cacaaccaaa 120
ccctatcatt ccacccatgg cccctcccaa atttcatgtc ctcacatttc aaaaccaatc 180
acaccatccc aacagtccct caaagtctta aatgatttca gcattaactc aaaagtccac 240
agtctaatgt ctcatctgag acaaggcaag tcctttccat ttatgagcct ataaaatcca 300
aagcaagtta gttacttcct agatacaatg gggggtacagg cattgggtaa atacagccat 360
tccaaatggg ataaattggt caaaacaaag aggctacagg cccatgagag tccaaaatcc 420
agtggggcag tcaaatctta aagctccaaa atgatctcct ttgactccac atctcacatc 480
caggtcacgc agatggaagg ggtgggttcc catggtcttg ggcagctctg cccctgtacc 540
tttgcagggt acagcctccc tctcagctgc tttcatgggc tggcattgag tgtctgcagc 600
ttttccaggt acacggtgca agctgtcggt ggatctacca ttctggggtc tggaggacct 660
cttctcacag ctccactagg tggtgcccca gtagggactg tgtgtggggt ctctgacccc 720
acatttccct tctgcactgc cctggcagag gatctccatg agggccctgc tcctgcagca 780
aacttctgac tgggcatcca ggcatttccg cacatcctct ttaatctagg cgaaggtttc 840
caaaccccaa ttcttgactt ctgtgcactc gcagtctcaa caccacatgg aagctgtcaa 900
ggcttggggc ttgcactccc cgaagctaca gcccaagctc taccttgcct cccgtcagtc 960
atggttggga gtggctggga tgcagggcac caagtcccta ggctgcacac agcatgagga 1020
ccccgggcct ggccaacaaa accatttttt cctgatacct ctggacctgt gatgggaggg 1080
gttgccataa agacctctga catgccctgg agacattttc cccattgtct tgggaattag 1140
catttggctc ctgttactca tgcaaatttc tgcagccagc ttgaatttct cctcagaaaa 1200
tgggaatttt tcttttctat cacattgtca ggctgcaaat tttccgaact tttatgctct 1260
gcttccctta taaaactgaa tgtctttaac agcacccaag tcacctcttg aatgctttgc 1320
tgcttagaaa tttctcctgc cagatactct aaatcatctc tctgaagttc aaagttctac 1380
aaatatctcg tgcaggggca aaatgccgcc agtatctttg ctaaaacata acaagagtcc 1440
cctttgctcc agttcccaac aagttcctca tttccgtctg agaccacctc agcctatgga 1500
ctttattgtc cacagtgcta tcagcatttt gggcaaagcc attcaacaag tctctaggaa 1560
gttccaaact ttcccacatt tgcctgtctt cttctgagcc ctccaaactg ttccaaaccc 1620
tgcctgttac ccagttccaa agtcacatac ccatttttga gtatctacgg cagcacccca 1680
ctctactggt accaatttag ccactgaagt agttggagaa cagaagtaat agactctggt 1740
ttacattgta aaagcttctc tgtggctgct gtgtgaagaa aatatatgag aatgaagccc 1800
caagatgaag cagggacaca gttgcagtgg ttagagtaag aaatgctgct ggctggcact 1860
gaagtgatag cctggaggtt tgtgtgtgca catgcatgtg tatgtgtttt acgatagtag 1920
gcccaacaga tactgtaatc cacacttgtt ttttttttt gagacagagt ctcacctgtt 1980
gcctagacta gaatgcagtg gcacaatctt ggctcactac aacctccacc tcccaggttc 2040
aaacaatcct tgtgcttcag cctcccgagt agttgggatt acaggtgtgt gccaccgtgc 2100
ccagctatat tttttgtatt tttagcagag atgggatttt gccacattgg ccaggctggt 2160
cttgaactcc tggcctcaag caatcctccc accttagcct cccaaagtgc tgagccacca 2220
cacctggccg caactgattt ttaatcatga aatgacacat acatttaaaa aacccaatac 2280
```

```
ctataatatt cctggctagt actcttcaca tctatatcat caaaaacaaa gaaagtatgt 2340
gaaactgaca cagccaaggg gagactaagg agacataaca attaactgta atgtggtatt 2400
ctggagggga tcctggaaca gaaaaagaca ttaggcaaaa aactaaagaa atctgaataa 2460
aatgtggatg tcagttaata ataatgtatc atattagtcc agtaattgta acaaatatac 2520
cacaataatg aaagccatta attataggga aaatggaggg gttaatatgg gtggctggct 2580
tttgctattt ctagcagctc cattttatct gcaaaagaca aacattcatt aagtcccaaa 2640
aaggtaaaga atgacaaatt aagcatgtat cttattagta agagtaatat aaagatgctc 2700
actcctattt ataaatattt gacaatcatg ttaaggccac aaaagagaaa aaagggtagg 2760
ggcaaaaaac gcaaagagaa aggagttagt atctttctc ccgcactcat tagctattaa 2820
aagaggatgt ttgtttaaag ctgctcagag ctggtaaact aatgttaagt cactaacggg 2880
aatttaaaag gtttcattaa gaactgcctg cactagattc ctccaccctg agacattaaa 2940
caatcacgat aaacctcctg agtggtaaga acttgtccat ttaaaaacag gctatagatt 3000
gtatcatgca gttttatcta ctaatcggct aatatcccgc caaaaacaaa aaaccccaaa 3060
gggatgaaag tttcatccat caaaggaaac aac                             3093
```

<210> 16
<211> 3099
<212> DNA
<213> Homo sapiens

<400> 16

```
attcactatc acaagaacag cacgggtaag acctgtcccc atgattcagt tacctcccac 60
tgggtccctc ccacaacgca tgggaattca ggatgagatt tgggtgggga cacaaccaaa 120
ccctatcatt ccacccatgg cccctcccaa atttcatgtc ctcacatttc aaaaccaatc 180
acaccatccc aacagtccct caaagtctta aatgatttca gcattaactc aaaagtccac 240
agtctaatgt ctcatctgag acaaggcaag tcctttccat ttatgagcct ataaaatcca 300
aagcaagtta gttacttcct agatacaatg gggtacagg cattgggtaa atacagccat 360
tccaaatggg ataaattggt caaaacaaag aggctacagg cccatgagag tccaaaatcc 420
agtggggcag tcaaatctta aagctccaaa atgatctcct ttgactccac atctcacatc 480
caggtcacgc agatggaagg ggtgggttcc catggtcttg ggcagctctg cccctgtacc 540
tttgcagggt acagcctccc tctcagctgc tttcatgggc tggcattgag tgtctgcagc 600
ttttccaggt acacggtgca agctgtcggt ggatctacca ttctggggtc tggaggacct 660
cttctcacag ctccactagg tggtgcccca gtagggactg tgtgtggggt ctctgacccc 720
acatttccct tctgcactgc cctggcagag gatctccatg agggccctgc tcctgcagca 780
aacttctgac tgggcatcca ggcatttccg cacatcctct ttaatctagg cgaaggtttc 840
caaaccccaa ttcttgactt ctgtgcactc gcagtctcaa caccacatgg aagctgtcaa 900
ggcttggggc ttgcactccc cgaagctaca gcccaagctc taccttgcct cccgtcagtc 960
atggttggga gtggctggga tgcagggcac caagtcccta ggctgcacac agcatgagga 1020
ccccgggcct ggccaacaaa accatttttt cctgatacct ctggacctgt gatgggaggg 1080
gttgccataa agacctctga catgccctgg agacattttc cccattgtct tgggaattag 1140
catttggctc ctgttactca tgcaaatttc tgcagccagc ttgaatttct cctcagaaaa 1200
tgggaatttt tcttttctat cacattgtca ggctgcaaat tttccgaact tttatgctct 1260
gcttccctta taaaactgaa tgtctttaac agcacccaag tcacctcttg aatgctttgc 1320
tgcttagaaa tttctcctgc cagatactct aaatcatctc tctgaagttc aaagttctac 1380
aaatatctcg tgcaggggca aaatgccgcc agtatctttg ctaaaacata acaagagtcc 1440
cctttgctcc agttcccaac aagttcctca tttccgtctg agaccacctc agcctatgga 1500
ctttattgtc cacagtgcta tcagcatttt gggcaaagcc attcaacaag tctctaggaa 1560
gttccaaact ttcccacatt tgcctgtctt cttctgagcc ctccaaactg ttccaaaccc 1620
tgcctgttac ccagttccaa agtcacatac ccatttttga gtatctacgg cagcacccca 1680
ctctactggt accaatttag ccactgaagt agttggagaa cagaagtaat agactctggt 1740
ttacattgta aaagcttctc tgtggctgct gtgtgaagaa aatatatgag aatgaagccc 1800
caagatgaag cagggacaca gttgcagtgg ttagagtaag aaatgctgct ggctggcact 1860
gaagtgatag cctggaggtt tgtgtgtgca catgcatgtg tatgtgtttt acgatagtag 1920
gcccaacaga tactgtaatc cacacttgtt tttttttttt ttttgagaca gagtctcacc 1980
tgttgcctag actagaatgc agtggcacaa tcttggctca ctacaacctc cacctcccag 2040
gttcaaacaa tccttgtgct tcagcctccc gagtagttgg gattacaggt gtgtgccacc 2100
gtgcccagct atattttttg tatttttagc agagatggga ttttgccaca ttggccaggc 2160
tggtcttgaa ctcctggcct caagcaatcc tcccacctta gcctcccaaa gtgctgagcc 2220
accacacctg gccgcaactg atttttaatc atgaaatgac acatacattt aaaaaaccca 2280
atacctataa tattcctggc tagtactctt cacatctata tcatcaaaaa caaagaaagt 2340
atgtgaaact gacacagcca aggggagact aaggagacat aacaattaac tgtaatgtgg 2400
tattctggag gggatcctgg aacagaaaaa gacattaggc aaaaaactaa agaaatctga 2460

ataaaatgtg gatgtcagtt aataataatg tatcatatta gtccagtaat tgtaacaaat 2520
ataccacaat aatgaaagcc attaattata gggaaaatgg aggggttaat atgggtggct 2580
ggcttttgct atttctagca gctccatttt atctacaaaa gacaaacatt cattaagtcc 2640
caaaaaggta aagaatgaca aattaagcat gtatcttatt agtaagagta atataaagat 2700
gctcactcat atttataaat atttgacaat gatgttaagg ccagaaaaga gaaaaaaggg 2760
taggggcaaa aaacgcaaag agaaaggagt tagtatcttt tctcccgcac tcattagcta 2820
ttaaaagagg atgtttgttt aaagctgctc agagctggta aactaatgtt aagtcactaa 2880
cgggaattta aaaggtttca ttaagaactg cctgcactag attcctccac cctgagacat 2940
taaacaatca cgataaacct cctgagtggt aagaacgtgt ccatttaaaa acaggctata 3000
gattgtcatg cagttttatc tactaatcgg ctaatgcacc gccaaaaaca aacaaaaaaa 3060
cccaaaggga tgaaagtttc atccatcaaa ggaaacaac                       3099
```

<210> 17
<211> 3099
<212> DNA
<213> Homo sapiens

<400> 17

```
attcactatc acgagaacag cacgggtaag acctgtcccc atgattcagt tacctcccac 60
tgggtccctc ccacaacgca tgggaattca ggatgagatt tgggtgggga cacaaccaaa 120
ccctatcatt ccacccatgg cccctcccaa atttcatgtc ctcacatttc aaaaccaatc 180
acaccatccc aacagtccct caaagtctta aatgatttca gcattaactc aaaagtccac 240
agtctaatgt ctcatctgag acaaggcaag tcctttccat ctatgagcct ataaaatcca 300
aagcaagtta attacttcct agatacaatg gggtacagg cattgggtaa atacagccat 360
tccaaatggg ataaattggt caaaacaaag aggctacagg cccatgagag tccaaaatcc 420
agtggggcag tcaaatctta aagctccaaa atgatctcct cttgactcca catctcacat 480
ccaggtcacg cagatggaag ggtgggttc ccatggtctt gggcagctct gcccctgtac 540
ctttgcaggg tacagcctcc ctctcagctg ctttcatggg ctggcattga gtgtctgcaa 600
cttttccagg tacacggtgc aagctgtcgg tggatctacc attctggggt ctggaggacc 660
tcttctcaca gctccactag gtggtgcccc agtagggact gtgtgtgggg tctctgaccc 720
cacatttccc ttctgcactg ccctggcaga ggatctccat gagggccctg cccctgcagc 780
aaacttctgc ctgggcatcc aggcatttcc gcacatcctc tttaatctag gcgaaggttt 840
ccaaacccca gttcttgact tctgtgcact cgcagtctca acaccacatg gaagctgtca 900
aggcttgggg cttgcactcc ccgaagctac agcccaagct ctaccttgcc tcctgtcagt 960
catggttggg agtggctggg atgcagggca ccaagtccct aggctgcaca cagcatgagg 1020
accccgggcc tggccaacaa aaccattttt tcctgatatc tctggacctg tgatgggagg 1080
ggttgccata aagacctctg acatgccctg gagacatttt ccccattgtc ttgggaatta 1140
gcatttggct cctgttactc atgcaaattt ctgcagccag cttgaatttc tcctcagaaa 1200
atgggaattt ttcttttcta tcacattgtc aggctgcaaa ttttccgaac ttttatgctc 1260
tgcttccctt ataaaactga atgtctttaa cagcacccaa gtcacctctt gaatgctttg 1320
ctgcttagaa atttctcctg ccagatactc taaatcatct ctctgaagtt caaagttcta 1380
caaatatctc gtgcaggggc aaaatgccgc cagtatcttt gctaaaacat aacaagagtc 1440
ccctttgctc cagttcccaa caagttcctc atttccgtct gagaccacct cagcctatgg 1500
actttattgt ccacagtgct atcagcattt tgggcaaagc cattcaacaa gtctctagga 1560
agttccaaac tttcccacat ttgcctgtct tcttctgagc cctccaaact gttccaaacc 1620
ctgcctgtta cccagttcca aagtcacata cccatttttg agtatctacg gcagcacccc 1680
actctactgg taccaattta gccactgaag tagttggaga acagaagtaa tagactctgg 1740
tttacattgt aaaagcttct ctgtggctgc tgtgtgaaga aaatatatga gaatgaagcc 1800
ccaagatgaa gcagggacac agttgcagtg gttagagtaa gaaatgctgc tggctggcac 1860
tgaagtgata gcctggaggt ttgtgtgtgc acatgcatgt gtatgtgttt tacgatagta 1920
ggcccaacag atactgtaat ccacacttgt tttttttttt tttttgagac agagtctcac 1980
ctgttgccta gactagaatg cagtggcaca atcttggctc actacaacct ccacctccca 2040
ggttcaaaca atccttgtgc ttcagcctcc cgagtagttg ggattacagg tgtgtgccac 2100
cgtgcccagc tatatttttt gtatttttag cagagatggg attttgccac attggccagg 2160
ctggtcttga actcctggcc tcaagcaatc ctcccacctt agcctcccaa agtgctgagc 2220
caccacacct ggccgcaact gatttttaat catgaaatga cacatacatt taaaaaaccc 2280
aatacctata atattcctgg ctagtactct tcacatctat atcatcaaaa acaaagaaag 2340
tatgtgaaac tgacacagcc aaggggagac taaggagaca taacaattaa ctgtaatgtg 2400
gtattctgga ggggatcctg gaacagaaaa agacattagg caaaaaacta aagaaatctg 2460
aataaaatgt ggatgtcagt taataataat gtatcatatt agtccagtaa ttgtaacaaa 2520
tatacccaat aatgaaagcc attaattata gggaaaatgg aggggttaat atgggtggct 2580
ggcttttgct atttctagca gctccatttt atctacaaaa gacaaacatt cattaagtcc 2640

caaaaaggta aagaatgaca aattaagcat gtatcttatt agtaagagta atataaagat 2700
gctcactcat atttataaat atttgacaat gatgttaagg ccagaaaaga gaaaaaaggg 2760
taggggcaaa aaacgcaaag agaaaggagt tagtatcttt tctcccgcac tcattagcta 2820
ttaaaagagg atgtttgttt aaagctgctc agagctggta aactaatgtt aagtcactaa 2880
cgggaattta aaaggtttca ttaagaactg cctgcactag attcctccac cctgagacat 2940
taaacaatca cgataaacct cctgagtggt aagaacgtgt ccatttaaaa acaggctata 3000
gattgtcatg cagttttatc tactaatcgg ctaatgcacc gccaaaaaca aacaaaaaaa 3060
cccaaaggga tgaaagtttc atccatcaaa ggaaacaac                        3099
```

<210> 18
<211> 9241

<212> DNA
<213> Homo sapiens

<400> 18

```
ctagaaaaca ctttgtcatt ttagaggtgt tatccaatgt tcgcgcaggc actggagtca 60
gagaaaatgg agttgaatcc tttctctgcc actctttgag gagaatctca ccatttatta 120
tgcactgtag aatacaacaa taaaatacag ccatgtacca cataacaaca tcttggtaaa 180
caacagactg catatatgat ggtggtcatc cagtaagcta aggttaattt attattattc 240
cctttttttt ttcttttttt tgagatgtag tcttactctg tcacccaggc tagagtgcaa 300
tggcaccatc ttggctcact gcaacctctg cctcctgggt tcaagcgaat ctcctgcctc 360
agcctccgaa gtagctggga attacaggca cccaccacat ctggctaatt ttttgtattt 420
ttagtaaaga tggggtttca ccatgttggc caggctgatc tcaaactcct gacctcaagt 480
gatctgcctg cctcggcctc ccaaagtgct gggaccatag gcctgagcca ctgtgcccgg 540
ccttgtttgc tttttaaaca gttaacagtg tgctcataga aactgctttg acatgactgc 600
aatcatgtgc ttcatagaaa cttaattaga ttataccact agagtcttca gatttttata 660
cttttttttt tgaaacggag tctcactctg tcaccaggct ggagtgcagt gccgcaatct 720
cagctcgccg caacctccgc ctcccaggtt caagtgattc tcctgcctca gcctcccgag 780
tagctgggat tacaagtgca cactaccacg cccagctaat ttttgcattt ttactagaca 840
gggtttcacc atgttggcta ggatagtttc accaggatct cttggcctca tgatcagcct 900
gcctcggcct cccaaagtgc tgggattaca ggtgtgagcc accgtgccca gcctatactt 960
ccctttttga ataccatttg gcgttttgaa gaattaacag ctttgtgaac gtggcagtgc 1020
ttgtgattca ggcttccact gagaccaagg ggagaacctg gttgcaggac aaacagacgg 1080
acagcgtgtg gcagtgttta aatgctcttc tgaaggctga tacgacagct ctctgtgcac 1140
tgattgtata cgcatcccaa gattatatta ttgttttcta ttgctatgtg tcacactttg 1200
ccaaacagga tgtggaaaat gaataagcgg ttttcttagg cacttcttaa cagacaattg 1260
gtcaaaatga actccattgc ttaagaaaca cataaacacc atttagtcac tgaatatagc 1320
tatatgtatg gttgctacta tggggaatct tgttttgcca attttctttg aaaattctgg 1380
cagaccaagg ttcttttttgt ttacacaata cttgaaaaat aaaaatgaac aagccaacaa 1440
actaccaagt tttcacttac ataaatgtag ttacatacag aaaatgtgac tgtgaatttt 1500
ttctaggact tttaaactat aagcactatt tgcacgaaag agaaccaatc tatcaattac 1560
aaactcacat aatttacag atttttttt ccctacacag cacataaaac agaaggaatt 1620
tgaagccacc ctccaaacac aggggaagga ggctgtgtgt atatcctcat tgtctttcac 1680
attctaaggt ggttccactc agtgactgaa atccttaagt gttgtattag tcggcttggg 1740
ctaccataac agcagcttaa actgttgtta agccactcag acttaaacaa cagaaattta 1800
tttccttata gttctggagg ctggaagttc aaggtgccgg caaggctggt ttctggtgag 1860
acctctctcc ctgtcttgca gatggctgcc tcctccctgt gtcctcatag agcctgtcct 1920
ctgcttttac acttctggtg tcatcttcct tttttttttt tttttgagac agagtctcgc 1980
tctatcgccc aggctggagt gcagtggccc gatcgatctc ggctcactgc aacctctgcc 2040
tcccaggttc aagcaattct cctgcctcag cctcccgagt agctgggact acaggtgccc 2100
gccatcatgt ctggctaatt tttgtatttt tagtagagac agggtttcac catattggcc 2160
aggctggtct ccaactcctg accttgtcat ctgcctgcct cggcctccca aagtgctagg 2220
attacaggcg tgagccaccg cacccggcct ctttctcttc ttataaggac accagtccta 2280
ttagattagg gctccacccct catgacctca tttgacctta actattattt ctttaaagca 2340
cctatttcca aatatagtca ctttaggggt tagggcttca aaatatgaat ctgagggaga 2400
tcaattcagt aaatagcagt agtcattaac ggacaatata tacaaagata atttcgtgat 2460
tactgtcctt atgcataaat gtcctcagtg ttccactgcc tttatccaga tttactatca 2520
caaagacttt gctctgagaa aaatgtgatt tctttctttt tttttttttt ttgagacaga 2580
gtctcactct gtcacccagg ctggagtgca gtggtgcaat ctcggctcac tgcaatctcc 2640
gcctcccagg ttcacgccat tctcttgcct cagtctcccg agtagctggg cctacaggcg 2700
cccgccaccc tgcccagcta attttttgta tttttagtag agacggggtt tcaccatgtt 2760
agccaggatg gtctcaatct cctgacctcg tgatccacct gcctcagcct cccaaagtgc 2820
```

tgggattaca ggcatgagcc accgcgccca gcagattttt tttttttttt tttttttttg 2880
agatggagtc ttgctgtgtt gcccagcctg gagtgcagtg ttatgatttt ggctcactgc 2940
aacctctgtc taccatgttc aagcgattct cccacctctg cctcccgtgt agctgggatc 3000
acaggcacac gccaccacac ctagctactt tttgtatttt tagtagaaat ggggtttcac 3060
catgttggcc aggatggtcc cgaactcctg acctcaagtg atcctcctgc ctcggcctcc 3120
caaagtgctg ggattacagg tgtgagccac tgtgcctggc caaaaatgtg atttcttatt 3180
tcccacattg ccaattccat ttcaattaac tataatagct atgtctattg agcactcaag 3240
tgtattctag aaactgttcc tgattctggg gatatatcca tgaatcaact atagtccctg 3300
ttattaagta atctgtagtc tgactaaacc attagaaatt taaaaaatgg ctactttcaa 3360
agacatcttg gagttcagga gtcccacact gcgaaccata ttacctaata atccaacctg 3420
cttgtaattc acttatttaa ccaatattta ttgagtgcca actttgagcc taagatacag 3480
cagtaaacaa atggataaag tccctgtcct catgaaactt gtattctaat ggaagaaaca 3540
gaaaacaaac agatatagga tgtaatatca ggtagggata aatactttga attcaaacaa 3600
aagtatacgt agtcagggtt cgccaaagag acacagccaa tcggatacat agatatataa 3660
aagagggttt atgagttaga aagggctcac atgattacag aggctgagaa gtcccacagc 3720
agattgtctg caagctggag acccagggat actggtagca tggctcagtc caagtcccaa 3780
agcctcagaa tcaggaaagc tgatgatata attcttagcc caaaggcctt agaaccccag 3840
cggtgacgga aaggctgatg taggtcctgg agtcctgaga cccaacagcc tgggatcctg 3900
aaatccaagg gcaggaatgg aagcgtgtat tccagctcca agagagtaag accaatttgc 3960
ctttcttccg tttttgtttc aagccacctg cacattgagg gcggatggtt ccctcttagt 4020
ccattcagtc atatatcaat ctcttctgga aataccctca cagacacact aacaaataat 4080
gcctttccag ttctctaggt attctttaat ccagtcaagc tgacacctaa aattaaccat 4140
cacaaaagtt aaggagaaag aagacaactt gtagggggagg ctgctatgca agacagtgtg 4200
tgaaggaagg gctctctgaa gaggttaata tctgagcaga gacttgaatg aagtgaagaa 4260
gtgagccatg tgggtatggg gaatacaact tccaggtaga gaagacaagt gtggtgtgta 4320
tcagggtcag caaagaagcc atgtgacaga gaagggtggg ccagggagag acggataagt 4380
gatctaactc ctgaggaggt ggcctggcca ggagctagag catgaagatc tcgtaggact 4440
ttattctgca aggtgaaaag ccattgtatt agtctgttca caaacccgag actaggcaat 4500
ttacaaaaga aagagaggtt taatggactt acagttccac atggctgggg aggcctcaca 4560
atcatggcga aaggcaatga ggagcaagtc acgtcttacg tggatggcag gcaaagacaa 4620
agacagcttg tgcagagaaa ctcccccctta tagagccatc agatcctgtt agacttattc 4680
actatcacaa gaacagcacg ggtaagacct gtccccatga ttcagttacc tcccactggg 4740
tccctcccac aacgcatggg aattcaggat gagatttggg tggggacaca accaaaccct 4800
atcattccac ccatggcccc tcccaaattt catgtcctca catttcaaaa ccaatcacac 4860
catcccaaca gtccctcaaa gtcttaaatg atttcagcat taactcaaaa gtccacagtc 4920
taatgtctca tctgagacaa ggcaagtcct ttccatttat gagcctataa aatccaaagc 4980
aagttagtta cttcctagat acaatggggg tacaggcatt gggtaaatac agccattcca 5040
aatgggataa attggtcaaa acaaagaggc tacaggccca tgagagtcca aaatccagtg 5100
gggcagtcaa atcttaaagc tccaaaatga tctcctttga ctccacatct cacatccagg 5160
tcacgcagat ggaaggggtg ggttcccatg gtcttgggca gctctgcccc tgtacctttg 5220
cagggtacag cctccctctc agctgctttc atgggctggc attgagtgtc tgcagctttt 5280
ccaggtacac ggtgcaagct gtcggtggat ctaccattct ggggtctgga ggacctcttc 5340
tcacagctcc actaggtggt gccccagtag ggactgtgtg tggggtctct gaccccacat 5400
ttcccttctg cactgccctg gcagaggatc tccatgaggg ccctgctcct gcagcaaact 5460
tctgactggg catccaggca tttccgcaca tcctctttaa tctaggcgaa ggtttccaaa 5520
ccccaattct tgacttctgt gcactcgcag tctcaacacc acatggaagc tgtcaaggct 5580
tggggcttgc actccccgaa gctacagccc aagctctacc ttgcctcccg tcagtcatgg 5640
ttgggagtgg ctgggatgca gggcaccaag tccctaggct gcacacagca tgaggacccc 5700
gggcctggcc aacaaaacca ttttttcctg atacctctgg acctgtgatg ggaggggttg 5760
ccataaagac ctctgacatg ccctggagac attttcccca ttgtcttggg aattagcatt 5820
tggctcctgt tactcatgca aatttctgca gccagcttga atttctcctc agaaaatggg 5880
aatttttctt ttctatcaca ttgtcaggct gcaaatttc cgaactttta tgctctgctt 5940
cccttataaa actgaatgtc tttaacagca cccaagtcac ctcttgaatg ctttgctgct 6000
tagaaatttc tcctgccaga tactctaaat catctctctg aagttcaaag ttctacaaat 6060
atctcgtgca ggggcaaaat gccgccagta tctttgctaa aacataacaa gagtcccctt 6120
tgctccagtt cccaacaagt tcctcatttc cgtctgagac cacctcagcc tatggacttt 6180
attgtccaca gtgctatcag cattttgggc aaagccattc aacaagtctc taggaagttc 6240
caaactttcc cacatttgcc tgtcttcttc tgagccctcc aaactgttcc aaaccctgcc 6300
tgttacccag ttccaaagtc acatacccat ttttgagtat ctacggcagc accccactct 6360
actggtacca atttagccac tgaagtagtt ggagaacaga agtaatagac tctggtttac 6420
attgtaaaag cttctctgtg gctgctgtgt gaagaaaata tatgagaatg aagccccaag 6480
atgaagcagg gacacagttg cagtggttag agtaagaaat gctgctggct ggcactgaag 6540
tgatagcctg gaggtttgtg tgtgcacatg catgtgtatg tgttttacga tagtaggccc 6600

```
aacagatact gtaatccaca cttgtttttt tttttttttt gagacagagt ctcacctgtt 6660
gcctagacta gaatgcagtg gcacaatctt ggctcactac aacctccacc tcccaggttc 6720
aaacaatcct tgtgcttcag cctcccgagt agttgggatt acaggtgtgt gccaccgtgc 6780
ccagctatat tttttgtatt tttagcagag atgggatttt gccacattgg ccaggctggt 6840
cttgaactcc tggcctcaag caatcctccc accttagcct cccaaagtgc tgagccacca 6900
cacctggccg caactgattt ttaatcatga aatgacacat acatttaaaa aacccaatac 6960
ctataatatt cctggctagt actcttcaca tctatatcat caaaaacaaa gaaagtatgt 7020
gaaactgaca cagccaaggg gagactaagg agacataaca attaactgta atgtggtatt 7080
ctggagggga tcctggaaca gaaaaagaca ttaggcaaaa aactaaagaa atctgaataa 7140
aatgtggatg tcagttaata ataatgtatc atattagtcc agtaattgta acaaatatac 7200
ccaataatga aagccattaa ttatagggaa aatggagggg ttaatatggg tggctggctt 7260
ttgctatttc tagcagctcc attttatcta caaaagacaa acattcatta agtcccaaaa 7320
aggtaaagaa tgacaaatta agcatgtatc ttattagtaa gagtaatata aagatgctca 7380
ctcatattta taaatatttg acaatgatgt taaggccaga aaagagaaaa aagggtaggg 7440
gcaaaaaacg caaagagaaa ggagttagta tcttttctcc cgcactcatt agctattaaa 7500
agaggatgtt tgtttaaagc tgctcagagc tggtaaacta atgttaagtc actaacggga 7560
atttaaaagg tttcattaag aactgcctgc actagattcc tccaccctga gacattaaac 7620
aatcacgata aacctcctga gtggtaagaa cgtgtccatt taaaaacagg ctatagattg 7680
tcatgcagtt ttatctacta atcggctaat gcaccgccaa aaacaaacaa aaaaacccaa 7740
agggatgaaa gtttcatcca tcaaaggaaa caacagtcac cttggttccc atcccactca 7800
tatactgccg ccgtacatgt caatcagatg aacctgtgcg tatctcttaa cgacaattga 7860
cccacctttt taactgaagt gaagggggt tctgctccgc gaccacttcc tggatctctc 7920
ccttcaccct ctgtgttctt tcgggtgcac catcgggtca aagccgcagc aacgccgtct 7980
ctgtgtgatc gcatgtgccc ttctgcacac gaccttcccc cgagagtgac cagctaccgg 8040
acaggcacca aggagggcta ccgagcacct cccggaccgg cggctgcagg atcgcgagcg 8100
cctccgctag ggagaccgca cgttgcgcct gtgcttcctg cggtggcgcc ttctgcaagg 8160
agacctcgac cctgctccct ctccggggct ggatctgact ccttgacggt gattccagac 8220
gcgagaccca aactgacggc ttctagaaga ggggcgagcc cggccgcaag tctttcacgt 8280
agctaagtca tcgttgcttc cggcttctta ccgttctccc ctttgtaaac ggttacctcc 8340
cgaaaaccca ggctctcctc caacagtggt tctcaagcga ggcgatcttc cccgggaggg 8400
gatatttggc aaagtctggg ggcatttttg gttcactggg gctgctactt gcatccactg 8460
ggtagaggcg gggatgcag ctacacaacc tgcgaagcac gggacagcac cctccccaac 8520
ccagacagaa ttagccggcc caaaacctca gtagtgccca ggctgagaaa ccctgcctta 8580
aacaaacaac aaagaaaggc caagtcccat aagtgggtca ccgcgccgag actggggtcc 8640
acgggacacc ccagccacgc caagccggga agtccccgcc tcctggagct gaacccgccc 8700
ctctcccaga ggtggagctg cgggggcgg gaacaggcac ggagaaaata aacaagacta 8760
aaaagtcctg agtagcgctg tgtggccgca aacctgaacc caccttttgc accacgcggg 8820
acccggcact cttcctgcca cccacccctg agagggctgc gcggccgacc ccagtactag 8880
aaaacactcg tcacctcact caagacgggt acgaaggcca acggacgcct tcctttagaa 8940
cgctcagcac acagagcaac ttctcacgcc tactctcaaa tggcgtactc caaactagca 9000
ctcccgacgt ccagctgtga acccagagcg gcggaaagcc cctgaaccca gcgcccgggc 9060
atgcgcagac gcgttgttgt ggtgggcgtg gctccctccg gacccggcgc cccgccctcc 9120
gccccgtgtc cgcatgcgcg actgagccgg gtggatggta ctgctgcatc cgggtgtctg 9180
gaggctgtgg ccgttttgtt ttcttggcta aaatcggggg agtgaggcgg gccggcgcgg 9240
c                                                                 9241
```

<210> 19
<211> 9236
<212> DNA
<213> Homo sapiens

<400> 19

```
ctagaaaaca ctttgtcatt ttagaggtgt tatccaatgt tcgcgcaggc actggagtca 60
gagaaaatgg agttgaatcc tttctctgcc actctttgag gagaatctca ccatttatta 120
tgcactgtag aatacaacaa taaaatacag ccatgtacca cataacaaca tcttggtaaa 180
caacagactg catatatgat ggtggtcatc cagtaagcta aggttaattt attattattc 240
cctttttttt ttcttttttt tgagatgtag tcttactctg tcacccaggc tagagtgcaa 300
tggcaccatc ttggctcact gcaacctctg cctcctgggt tcaagcgaat ctcctgcctc 360
agcctccgaa gtagctggga attacaggca cccaccacat ctggctaatt ttttgtattt 420
ttagtaaaga tggggtttca ccatgttggc caggctgatc tcaaactcct gacctcaagt 480
gatctgcctg cctcggcctc ccaaagtgct gggaccatag gcctgagcca ctgtgcccgg 540
ccttgtttgc tttttaaca gttaacagtg tgctcataga aactgctttg acatgactgc 600
```

aatcatgtgc ttcatagaaa cttaattaga ttataccact agagtcttca gatttttata 660
cttttttttt tgaaacggag tctcactctg tcaccaggct ggagtgcagt gccgcaatct 720
cagctcgccg caacctccgc ctcccaggtt caagtgattc tcctgcctca gcctcccgag 780
tagctgggat tacaagtgca cactaccacg cccagctaat tttgcattt ttactagaca 840
gggtttcacc atgttggcta ggatagtttc accaggatct cttggcctca tgatcagcct 900
gcctcggcct cccaaagtgc tgggattaca ggtgtgagcc accgtgccca gcctatactt 960
ccctttttga ataccatttg gcgttttgaa gaattaacag ctttgtgaac gtggcagtgc 1020
ttgtgattca ggcttccact gagaccaagg ggagaacctg gttgcaggac aaacagacgg 1080
acagcgtgtg gcagtgttta aatgctcttc tgaaggctga tacgacagct ctctgtgcac 1140
tgattgcata cgcatcccaa gattatatta ttgttttcta ttgctatgtg tcacactttg 1200
ccaaacagga tgtggaaaat gaataagcgg ttttcttagg cacttcttaa cagacaattg 1260
gtcaaaatga actccattgc ttaagaaaca cataaacacc atttagtcac tgaatatagc 1320
tatatgtatg gttgctacta tggggaatct tgttttgcca attttctttg aaaattctgg 1380
cagaccaagg ttcttttttgt ttacacaata cttgaaaaat aaaaatgaac aagccaacaa 1440
actaccaagt tttcacttac ataaatgtag ttacatacag aaaatgtgac tgtgaatttt 1500
ttctaggact tttaaactat aagcactatt tgcacgaaag agaaccaatc tatcaattac 1560
aaactcacat aattttacag attttttttt ccctacacag cacataaaac agaaggaatt 1620
tgaagccacc ctccaaacac aggggaagga ggctgtgtgt atatcctcat tgtctttcac 1680
attctaaggt ggttccactc agtgactgaa atccttaagt gttgtattag tcggcttggg 1740
ctaccataac agcagcttaa actgttgtta agccactcag acttaaacaa cagaaattta 1800
tttccttata gttctggagg ctggaagttc aaggtgccgg caaggctggt ttctggtgag 1860
acctctctcc ctgtcttgca gatggctgcc tcctccctgt gtcctcatag agcctgtcct 1920
ctgcttttac acttctggtg tcatcttcct tttttttttt tttttgagac agagtctcgc 1980
tctatcgccc aggctggagt gcagtggccc gatcgatctc ggctcactgc aacctctgcc 2040
tcccaggttc aagcaattct cctgcctcag cctcccgagt agctgggact acaggtgccc 2100
gccatcatgt ctggctaatt tttgtatttt tagtagagac agggtttcac catattggcc 2160
aggctggtct ccaactcctg accttgtcat ctgcctgcct cggcctccca aagtgctagg 2220
attacaggcg tgagccaccg caccccggcct ctttctcttc ttataaggac accagtccta 2280
ttagattagg gctccaccct catgacctca tttgacctta actattattt ctttaaagca 2340
cctatttcca aatatagtca ctttaggggt tagggcttca aaatatgaat ctgagggaga 2400
tcaattcagt aaatagcagt agtcattaac ggacaatata tacaaagata atttcgtgat 2460
tactgtcctt atgcataaat gtcctcagtg ttccactgcc tttatccaga tttactatca 2520
caaagacttt gctctgagaa aaatgtgatt tctttctttt tttttttttt ttgagacaga 2580
gtctcactct gtcacccagg ctggagtgca gtggtgcaat ctcggctcac tgcaatctcc 2640
gcctcccagg ttcacgccat tctcttgcct cagtctcccg agtagctggg cctacaggcg 2700
cccgccaccc tgcccagcta atttttgta tttttagtag agacggggtt tcaccatgtt 2760
agccaggatg gtctcaatct cctgacctcg tgatccacct gcctcagcct cccaaagtgc 2820
tgggattaca ggcatgagcc accgcgccca gcagattttt tttttttttt tttttttttg 2880
agatggagtc ttgctgtgtt gcccagcctg gagtgcagtg ttatgatttt ggctcactgc 2940
aacctctgtc taccatgttc aagcgattct cccacctctg cctcccgtgt agctgggatc 3000
acaggcacac gccaccacac ctagctactt tttgtatttt tagtagaaat ggggtttcac 3060
catgttggcc aggatggtcc cgaactcctg acctcaagtg atcctcctgc ctcggcctcc 3120
caaagtgctg ggattacagg tgtgagccac tgtgcctggc caaaaatgtg atttcttatt 3180
tcccacattg ccaattccat ttcaattaac tataatagct atgtctattg agcactcaag 3240
tgtattctag aaactgttcc tgattctggg gatatatcca tgaatcaact atagtccctg 3300
ttattaagta atctgtagtc tgactaaacc attagaaatt taaaaaatgg ctactttcaa 3360
agacatcttg gagttcagga gtcccacact gcgaaccata ttacctaata atccaacctg 3420
cttgtaattc acttatttaa ccaatattta ttgagtgcca actttgagcc taagatacag 3480
cagtaaacaa atggataaag tccctgtcct catgaaactt gtattctaat ggaagaaaca 3540
gaaaacaaac agatatagga tgtaatatca ggtagggata aatactttga attcaaacaa 3600
aagtatacgt agtcagggtt cgccaaagag acacagccaa tcggatacat agatatataa 3660
aagagggttt atgagttaga aagggctcac atgattacag aggctgagaa gtcccacagc 3720
agattgtctg caagctggag acccagggat actggtagca tggctcagtc caagtcccaa 3780
agcctcagaa tcaggaaagc tgatgatata attcttagcc caaaggcctt agaaccccag 3840
cggtgacgga aaggctgatg taggtcctgg agtcctgaga cccaacagcc tgggatcctg 3900
aaatccaagg gcaggaatgg aagcgtgtat tccagctcca agagagtaag accaatttgc 3960
ctttcttccg tttttgtttc aagccacctg cacattgagg gcggatggtt ccctcttagt 4020
ccattcagtc atatatcaat ctcttctgga aatacctca cagacacact aacaaataat 4080
gcctttccag ttctctaggt attctttaat ccagtcaagc tgacacctaa aattaaccat 4140
cacaaaagtt aaggagaaag aagacaactt gtaggggagg ctgctatgca agacagtgtg 4200
tgaaggaagg gctctctgaa gaggttaata tctgagcaga gacttgaatg aagtgaagaa 4260
gtgagccatg tgggtatggg gaatacaact tccaggtaga gaagacaagt gtggtgtgta 4320
tcagggtcag caaagaagcc atgtgacaga gaagggtggg ccagggagag acggataagt 4380

gatctaactc ctgaggaggt ggcctggcca ggagctagag catgaagatc tcgtaggact 4440
ttattctgca aggtgaaaag ccattgtatt agtctgttca caaacccgag actaggcaat 4500
ttacaaaaga aagagaggtt taatggactt acagttccac atggctgggg aggcctcaca 4560
atcatggcga aaggcaatga ggagcaagtc acgtcttacg tggatggcag gcaaagacaa 4620
agacagcttg tgcagagaaa ctccccctta tagagccatc agatcctgtt agacttattc 4680
actatcacaa gaacagcacg ggtaagacct gtccccatga ttcagttacc tcccactggg 4740
tccctcccac aacgcatggg aattcaggat gagatttggg tggggacaca accaaaccct 4800
atcattccac ccatggcccc tcccaaattt catgtcctca catttcaaaa ccaatcacac 4860
catcccaaca gtccctcaaa gtcttaaatg atttcagcat taactcaaaa gtccacagtc 4920
taatgtctca tctgagacaa ggcaagtcct ttccatttat gagcctataa aatccaaagc 4980
aagttagtta cttcctagat acaatggggg tacaggcatt gggtaaatac agccattcca 5040
aatgggataa attggtcaaa acaaagaggc tacaggccca tgagagtcca aaatccagtg 5100
gggcagtcaa atcttaaagc tccaaaatga tctcctttga ctccacatct cacatccagg 5160
tcacgcagat ggaaggggtg ggttcccatg gtcttgggca gctctgcccc tgtacctttg 5220
cagggtacag cctccctctc agctgctttc atgggctggc attgagtgtc tgcagctttt 5280
ccaggtacac ggtgcaagct gtcggtggat ctaccattct ggggtctgga ggacctcttc 5340
tcacagctcc actaggtggt gccccagtag ggactgtgtg tggggtctct gaccccacat 5400
ttcccttctg cactgccctg gcagaggatc tccatgaggg ccctgctcct gcagcaaact 5460
tctgactggg catccaggca tttccgcaca tcctctttaa tctaggcgaa ggtttccaaa 5520
ccccaattct tgacttctgt gcactcgcag tctcaacacc acatggaagc tgtcaaggct 5580
tggggcttgc actccccgaa gctacagccc aagctctacc ttgcctcccg tcagtcatgg 5640
ttgggagtgg ctgggatgca gggcaccaag tccctaggct gcacacagca tgaggacccc 5700
gggcctggcc aacaaaacca tttttcctg atacctctgg acctgtgatg ggaggggttg 5760
ccataaagac ctctgacatg ccctggagac attttcccca ttgtcttggg aattagcatt 5820
tggctcctgt tactcatgca aatttctgca gccagcttga atttctcctc agaaaatggg 5880
aatttttctt ttctatcaca ttgtcaggct gcaaattttc cgaactttta tgctctgctt 5940
cccttataaa actgaatgtc tttaacagca cccaagtcac ctcttgaatg ctttgctgct 6000
tagaaatttc tcctgccaga tactctaaat catctctctg aagttcaaag ttctacaaat 6060
atctcgtgca ggggcaaaat gccgccagta tctttgctaa aacataacaa gagtcccctt 6120
tgctccagtt cccaacaagt tcctcatttc cgtctgagac cacctcagcc tatggacttt 6180
attgtccaca gtgctatcag cattttgggc aaagccattc aacaagtctc taggaagttc 6240
caaactttcc cacatttgcc tgtcttcttc tgagccctcc aaactgttcc aaaccctgcc 6300
tgttacccag ttccaaagtc acatacccat ttttgagtat ctacggcagc accccactct 6360
actggtacca atttagccac tgaagtagtt ggagaacaga agtaatagac tctggtttac 6420
attgtaaaag cttctctgtg gctgctgtgt gaagaaaata tatgagaatg aagccccaag 6480
atgaagcagg gacacagttg cagtggttag agtaagaaat gctgctggct ggcactgaag 6540
tgatagcctg gaggtttgtg tgtgcacatg catgtgtatg tgttttacga tagtaggccc 6600
aacagatact gtaatccaca cttgtttttt tttttgaga cagagtctca cctgttgcct 6660
agactagaat gcagtggcac aatcttggct cactacaacc tccacctccc aggttcaaac 6720
aatccttgtg cttcagcctc ccgagtagtt gggattacag gtgtgtgcca ccgtgcccag 6780
ctatattttt tgtattttta gcagagatgg gattttgcca cattggccag gctggtcttg 6840
aactcctggc ctcaagcaat cctcccacct tagcctccca aagtgctgag ccaccacacc 6900
tggccgcaac tgatttttaa tcatgaaatg acacatacat ttaaaaaacc caatacctat 6960
aatattcctg gctagtactc ttcacatcta tatcatcaaa aacaaagaaa gtatgtgaaa 7020
ctgacacagc caaggggaga ctaaggagac ataacaatta actgtaatgt ggtattctgg 7080
aggggatcct ggaacagaaa aagacattag gcaaaaaact aaagaaatct gaataaaatg 7140
tggatgtcag ttaataataa tgtatcatat tagtccagta attgtaacaa atataccaca 7200
ataatgaaag ccattaatta tagggaaaat ggaggggtta atatgggtgg ctggcttttg 7260
ctatttctag cagctccatt ttatctgcaa aagacaaaca ttcattaagt cccaaaaagg 7320
taaagaatga caaattaagc atgtatctta ttagtaagag taatataaag atgctcactc 7380
ctatttataa atatttgaca atcatgttaa ggccacaaaa gagaaaaaag ggtaggggca 7440
aaaaacgcaa agagaaagga gttagtatct tttctcccgc actcattagc tattaaaaga 7500
ggatgtttgt ttaaagctgc tcagagctgg taaactaatg ttaagtcact aacggaattt 7560
taaaaggttt cattaagaac tgcctgcact agattcctcc accctgagac attaaacaat 7620
cacgataaac ctcctgagtg gtaagaactt gtccatttaa aaacaggcta tagattgtat 7680
catgcagttt tatctactaa tcggctaata tcccgccaaa aacaaaaaac cccaaaggga 7740
tgaaagtttc atccatcaaa ggaaacaaca gtcaccttgg ttcccatctc actcatatac 7800
tgccgccgta catgtcaatc agatgaacct gtgcgtatct cttaatgaca attgacccac 7860
atttttgact gaagtgaaag gggttctgc tccgcgacca cttcctggat ctcccccctcc 7920
accctctgtg ttctttcggg tgcaccatcg ggtcaaagcc gcagcaacgc cgtctctgtg 7980
tgatcgcatg tgcccttctg cacacgacct tcccccgaga gtgaccagct accggacagg 8040
caccaaggag ggctaccgag cacctcccgg accggcggct gcaggatcgc gagcgcctcc 8100
gctagggaga ctgcacgttg cgcctgtgct tcctgcggtg gcgccttctg caaggagacc 8160

```
tcgaccctgc tccctctccg gggctggatc tgactccttg acggtgattc cagacgcgag 8220
acccaaactg acggcttcta gaagaggggc gagcccggcc gcaagtcttt cacgtagcta 8280
agtcatcgtt gcttccggct tcttaccgtt ctcccctttg taaacggtta cctcccgaaa 8340
acccaggctc tcctccaaca gtggttctca agcgaggcga tcttccccgg gaggggatat 8400
ttggcaaagt ctgggggcat tttggttca ctggggctgc tacttgcatc cactgggtag 8460
aggcggggga tgcagctaca caacctgcga agcacgggac agcaccctcc ccaacccaga 8520
cagaattagc cggcccaaaa cctcagtagt gcccaggctg agaaaccctg ccttaaacaa 8580
acaacaaaga aaagccaagt cccataagtg ggtcaccgcg ccgagactgg ggtccacggg 8640
acaccccagc cacgccaagc cgggaagtcc ccgcctcctg gagctgaacc cgcccctctc 8700
ccagaggtgg agctgcgggg ggcgggaaca ggcacggaga aaataaacaa gactaaaaag 8760
tcctgagtag cgctgtgtgg ccgcaaacct gaacccacct tttgcaccac gcgggacccg 8820
gcacgcttcc tgccacccac ccctgagagg gctgcgcggc cgaccccagt actagaaaac 8880
actcgtcacc tcaatcaaga cgggtacgaa ggccaacgga cgccttcctt tagaacgctc 8940
agcacacaga gcaacttctc acgcctactc tcaaatggcg tactccaaac tagcactccc 9000
gacgtccagc tgtgaaccca gagcggcgga aagcccctga acccagcgcc cgggcatgcg 9060
cagacgcgtt gttgtggtgg gcgtggctcc ctccggaccc ggcgccccgc cctccgcccc 9120
gtgtccgcat gcgcgactga gccgcggggg tggtactgct gcatccgggt gtctgaagat 9180
ccgatgaaat aacatatgca aaatgattgg gtccgtgatt ggcattccag aaatgg     9236
```

<210> 20
<211> 9238
<212> DNA
<213> Homo sapiens

<400> 20

```
aaacgctcat gacagcaaag tctccaatgt tcgcgcaggc actggagtca gagaaaatgg 60
agttgaatcc tttctctgcc actctttgag gagaatctca ccatttatta tgcactgtag 120
aatacaacaa taaaatacag ccatgtacca cataacaaca tcttggtaaa caacagactg 180
catatatgat ggtggtcatc cagtaagcta aggttaattt attattattc cttgtttttt 240
tttttttttt tttttttttg agatgtagtc ttactctgtc acccaggcta gagtgcaatg 300
gcaccatctt ggctcactgc aacctctacc tcctgggttc aagcaaatct cctgcctcag 360
cctccaaagt agctgggatt acaggcaccc accacatctg gctaattttt tgtattttta 420
gtaaagatgg ggtttcacca tgttggccag gctgatctca aactcctgac ctcaagtgat 480
ctgcccgcct cggcctccca aagtgctgga accacaggcc tgagccactg tgcccagcct 540
tggttgcttt tttaacagat aacagtgtgc tcatagaaac tgctttgaca tgactgcaat 600
catgtgcttc atagaaactt aattagatta taccactaga gtcttcagat ttttatactt 660
tttttttttg aaacggagtc tcactctgtc accaggctgg agtgcagtgc cgcaatctcg 720
gctcactgca acctccgcct cccaggttca agcaattctc ctgcctcagc ctcccgagta 780
gctgggatta caagtgcgca ctaccacacc cagctaattt ttgcattttt acttgacagg 840
gtttcaccat gttggctagg atagtttcac caggatctct tggcctcatg atcagcctgc 900
ctcggcctcc caaagtgctg ggattacagg tgtgagccac cgtgcccagc ctatacttcc 960
ctttttgaat accatttggc gttttgaaga attaacagct ttgtgaacgt ggcagtgctt 1020
gtgattcagg cttccattga gaccaagggg agaacctggt tgcaggacaa acagacggac 1080
agcgtgtggc agtgtttaaa tgctcttctg aaggctgata cgacagctct ctgtgcactg 1140
attgcatacg catcccaaga ttatattatt gttttctact gctatgtgtc acactttgcc 1200
aaacaggatg tggaaaatga ataagcggtt ttcttaggca cttcttaaca gacaattggt 1260
caaaatgaac tccattgctt aagaaacaca taaacaccat ttagtcactg aacatagcta 1320
tatgtatggt tgttactatg ggaaatcttg ttttgccaat tttctttgaa aattctggca 1380
gaccaaggtt ctttttgttt acataatact tgaaaaataa aaatgaacaa gctaacaaac 1440
taccaagttt tcacttacat aaatgtagtt gcatacagaa aatgtgactg tgaattaatt 1500
tttctaggac ttttaaacta taagcactat ttgcacaaaa gagaaccaat ctatcaatta 1560
caaactcaca taattttaca gatttttttt ttcctacaca gcacataaaa cagaaggaat 1620
ttgaagccac cctccaaaca caggggaagg aggctgtgtg tatatcctca ttgtctttca 1680
cattctaagg tggttccact cagtgactga aatccttaag cgttgtatta gtctgcttgg 1740
gctaccataa cagcagctta aactgttgtt tagccactca gacttaaaca acagaaattt 1800
atttccttat agttctggag gctggaagtt caaggtgccg gcaaggttgg tttctggtga 1860
gacctctctc cctgtcttgc agatggctgc ctcctccctg tgtcctcata gagcctgtct 1920
tctgctttta cacttctggt gtcatcttcc tttttttttt tttttttttt ttttgagaca 1980
gagtctcgct ctatcgccca ggctggagtg cagtggcccg atcgatctcg gctcactgca 2040
acctctgcct cccaggttca agcaattctc ctgcctcagc ctcccaagta gctgggacta 2100
caggtgcccg ccatcatgcc tggctaattt ttgtattttt agtagagaca gggtttcacc 2160
atattggcca ggctggtctc gaactcctga ccttgtcatc tgcctgcctc ggcctcccaa 2220
```

```
agtgctagga ttacaggcgt gagccaccgc acccggcctc ttcctcttct tataaggaca 2280
ccagtcctat tagattaggg ctccaccctc ataacctcat ttgaccttaa ctattatttc 2340
tttaaagcac ctatttccaa atatagtcac tttaggggtt agggcttcaa aagatgaatc 2400
tgagggagct caattcagta aatagcagta gtcattaatg gacaatgtat acaaagataa 2460
tttcgtgatt actgtcctta tgcataaacg tcctcagtgt tccactgcgt ttatccagat 2520
ttagtatcac aaagactttg ctctgagaaa aatgtgattt tttttttttt tttttttttg 2580
agacggagtc tcgctctgtt acccaggctg gagtgcagtg gcgcgatctc ggctcactgc 2640
aagctccgcc tcccgggttc acgccattct cctgcctcag cctccggagt agctgggact 2700
acaggcgccc gccactacgc ccggctaact tttttgtatt tttagtagag acggggtttc 2760
accatgttag ccaggatggt ctcaatctcc tgacctcgtg atccacctgc ctcagcctcc 2820
caaagtgctg ggattacagg catgagccac cgcgcccagc agattttttt tttttttttt 2880
gagatggagt cttgctctgt tgcccaacct ggagtgcagt gttatgattt tggctcactg 2940
caacctctac catgttcaag cgattctccc acctctgcct cccgtgtagc tgggatcaca 3000
ggcacacgcc accacaccta gctacttttt gtatttttag tagaaatggg gtttcaccat 3060
gttggccagg atggtcccga actcctgacc tcaagtgatc ctcctgcctc ggcctccaaa 3120
gtgctgggat tacaggtgtg agccactgtg cctggccaaa aatgtgattt cttatttccc 3180
acattgccaa ttccatttca attaactata atagctatgt ctattgagca ctcaagcgta 3240
ttctagaaac tgttcctgat tctggggata tatccatgaa tcaactatag tccctgttat 3300
taagtaatcc gtagtctgac taaaccatta gaaattaaaa aaaaatggct actttcaaag 3360
acatcttgga gttcaggagt cccacactgc gaaccatatt acctaataat ccaacctgct 3420
tgtaattcac ttatttaacc aatatttatt gagtgccaac tttgagccta agatacagca 3480
gtaaacaaat gcataaagtc cctgtcccca tgaaacttgt attctaatgg aaaaaacaga 3540
aaacaaacag atataggatg taatatcagg tagggataaa tactttgaat tcaaacaaaa 3600
gtatacgtag tcagggttcc ccagagagac acagccaatc gatacataga tatataagag 3660
agggtttatg agttagaaag ggctcacatg attacagagg ctgagaagtc ccacaacaga 3720
ttgtctgcaa gctggagacc cagggatact ggtagcatgg ctcagtccaa gtcccaaagt 3780
ctcagaatca ggaaagctga tgatataatt cttagcccaa aggccttaga accccagcgg 3840
tgacggaaag gctgatgtag gtcctggcgt cctgagaccc aacagcctgg gatcctgaaa 3900
tccaagggca ggaatggaag cgtgtattcc agctccaaga gagtaagacc aatttgcctt 3960
tcttccgttt ttgttccaag ccaactgcac gttgagggcg gatggttccc tcttagccca 4020
ttcagtcata tatcaatctc ttctggaaat accctcacag acacactaac aaataatgcc 4080
tttccagttc tctaggtatt ctttaatcca gtcaagctga cacctaaaat taaccatcac 4140
aaaagttaag gagaaagaag acaacttgta ggagaggctg ctatgcaaga cagtgtgtga 4200
aggaagggct ctctgaagag gttaatatct gagcagagac ttgaatgaag tgaagaagtg 4260
agccatgtgg gtatggggaa tacaacttcc aggtagagaa gacaagtgtg gtgtgtatca 4320
cggtcagcaa agaagccatg tgacagagaa gggtgggcca gggagagacg gataagtgat 4380
ctaactcctg aggaggtggc ctggccagga gctagagcat gaggatctcg taggatttta 4440
ttctgcaagg tgaaaagcca ttgtattagt ctgttcacaa accccagact aggcaattta 4500
caaaagaaag agaggtttaa tggacttaca gttccacatg gctggggagg cctcacaatc 4560
atggcgaaag gcaatgagga gcaagtcacg tcttacgtgg atggcaggca aagacaaaga 4620
cagcttgtgc agagaaactc ccccttatag agccatcaga tcctgttaga cttatcacta 4680
tcacgagaac agcacgggta agacctgtcc ccatgattca gttacctccc actgggtccc 4740
tcccacaacg catgggaatt caggatgaga tttgggtggg gacacaacca aaccctatca 4800
ttccacccat ggcccctccc aaatttcatg tcctcacatt tcaaaaccaa tcacaccatc 4860
ccaacagtcc ctcaaagtct taaatgattt cagcattaac tcaaaagtcc acagtctaat 4920
gtctcatctg agacaaggca agtcctttcc gtctatgagc ctataaaatc caaagcaagt 4980
taattacttc ctagatacaa tgggggtaca ggcattgggt aaatacagcc attccaaatg 5040
ggataaattg gtcaaaacaa agaggctaca ggcccatgag agtccaaaat ccagtggggc 5100
agtcaaatct taaagctcca aaatgatctc ctcttgactc cacatctcac atccaggtca 5160
tgcagatgga aggggtgggt tcccatggtc ttgggcagct ctgcccctgt acctttgcag 5220
ggtacagcct ccctctcagc tgctttcatg ggctggcatt gagtgtctgc aacttttcca 5280
ggtacacggt gcaagctgtc ggtggatcta ccattctggg gtctggagga cctcttctca 5340
cagctccact aggtggtgcc ccagtaggga ctgtgtgtgg ggtctctgac cccacatttc 5400
ccttctgcac tgccctggca gaggatctcc atgagggccc tgcccctgca gcaaacttct 5460
gcctgggcat ccaggcattt ccgcacatcc tctttaatct aggcgaaggt ttccaaaccc 5520
cagttcttga cttctgtgca ctcgcagtct caacaccaca tggaagctgt caaggcttgg 5580
ggcttgcact ccccgaagct acagcccaag ctctaccttg cctcctgtca gtcatggttg 5640
ggagtggctg ggatgcaggg caccaagtcc ctaggctgca cacagcatga ggaccccggg 5700
cctggccaac aaaaccattt tttcctgata tctctggacc tgtgatggga ggggttgcca 5760
taaagacctc tgacatgccc tggagacatt ttccccattg tcttgggaat tagcatttgg 5820
ctcctgttac tcatgcaaat ttctgcagcc agcttgaatt tctcctcaga aaatgggaat 5880
ttttcttttc tatcacattg tcaggctgca aattttccga acttttatgc tctgcttccc 5940
ttataaaact gaatgtcttt aacagcaccc aagtcacctc ttgaatgctt tgctgcttag 6000
```

```
aaatttctcc tgccagatac tctaaatcat ctctctgaag ttcaaagttc tacaaatatc 6060
tcgtgcaggg gcaaaatgcc gccagtatct ttgctaaaac ataacaagag tccccttttgc 6120
tccagttccc aacaagttcc tcatttccgt ctgagaccac ctcagcctat ggactttatt 6180
gtccacagtg ctatcagcat tttgggcaaa gccattcaac aagtctctag gaagttccaa 6240
actttcccac atttgcctgt cttcttctga gccctccaaa ctgttccaaa ccctgcctgt 6300
tacccagttc caaagtcaca tacccatttt tgagtatcta cggcagcacc ccactctact 6360
ggtaccaatt tagccactga agtagttgga gaacagaagt aatagactct ggtttacatt 6420
gtaaaagctt ctctgtggct gctgtgtgaa gaaaatatat gagaatgaag ccccaagatg 6480
aagcagggac acagttgcag tggttagagt aagaaatgct gctggctggc actgaagtga 6540
tagcctggag gtttgtgtgt gcacatgcat gtgtatgtgt tttacgatag taggcccaac 6600
agatactgta atccacactt gttttttttt tttttttgag acagagtctc acctgttgcc 6660
tagactagaa tgcagtggca caatcttggc tcactacaac ctccacctcc caggttcaaa 6720
caatccttgt gcttcagcct cccgagtagt tgggattaca ggtgtgtgcc accgtgccca 6780
gctatatttt ttgtattttt agcagagatg ggattttgcc acattggcca ggctggtctt 6840
gaactcctgg cctcaagcaa tcctcccacc ttagcctccc aaagtgctga gccaccacac 6900
ctggccgcaa ctgattttta atcatgaaat gacacataca tttaaaaaac ccaataccta 6960
taatattcct ggctagtact cttcacatct atatcatcaa aaacaaagaa agtatgtgaa 7020
actgacacag ccaaggggag actaaggaga cataacaatt aactgtaatg tggtattctg 7080
gaggggatcc tggaacagaa aaagacatta ggcaaaaaac taaagaaatc tgaataaaat 7140
gtggatgtca gttaataata atgtatcata ttagtccagt aattgtaaca aatataccca 7200
ataatgaaag ccattaatta tagggaaaat ggaggggtta atatgggtgg ctggcttttg 7260
ctatttctag cagctccatt ttatctacaa aagacaaaca ttcattaagt cccaaaaagg 7320
taaagaatga caaattaagc atgtatctta ttagtaagag taatataaag atgctcactc 7380
atatttataa atatttgaca atgatgttaa ggccagaaaa gagaaaaaag ggtaggggca 7440
aaaaacgcaa agagaaagga gttagtatct tttctcccgc actcattagc tattaaaaga 7500
ggatgtttgt ttaaagctgc tcagagctgg taaactaatg ttaagtcact aacgggaatt 7560
taaaaggttt cattaagaac tgcctgcact agattcctcc accctgagac attaaacaat 7620
cacgataaac ctcctgagtg gtaagaacgt gtccatttaa aaacaggcta tagattgtca 7680
tgcagtttta tctactaatc ggctaatgca ccgccaaaaa caaacaaaaa aacccaaagg 7740
gatgaaagtt tcatccatca aaggaaacaa cagtcacctt ggttcccatc ccactcatat 7800
actgccgccg tacatgtcaa tcagatgaac ctgtgcgtat ctcttaatga caattgaccc 7860
acctttttaa ctgaagtgaa gggggttct gctccgcgac cacttcctgg atctctccct 7920
tcaccctctg tgttctttcg ggtgcaccat cgggtcaaag ccgcagcaac gccgtctctg 7980
tgtgatcgca tgtgcccttc tgcacacgac cttcccccga gagtgaccag ctaccggaca 8040
ggcaccaagg agggctaccg agcacctccc ggaccggcgg ctgcaggatc gcgagcgcct 8100
ccgctaggga gaccgcacgt tgcgcctgtg cttcctgcgg tggcgccttc tgcaaggaga 8160
cctcgaccct gctccctctc cggggctgga tctgactcct tgacggtgat tccagacgcg 8220
agacccaaac tgacggcttc tagaagaggg gcgagcccgg ccgcaagtct ttcacgtagc 8280
taagtcatcg ttgcttccgg cttcttaccg ttctcccctt tgtaaacggt tacctcccga 8340
aaacccaggc tctcctccaa cagtggttct caagcgaggc gatcttcccc gggaggggat 8400
atttggcaaa gtctgggggc atttttggtt cactggggct gctacttgca tccactgggt 8460
agaggcgggg gatgcagcta cacaacctgc gaagcacggg acagcaccct ccccaaccca 8520
gacagaatta gccggcccaa aacctcagta gtgcccaggc tgagaaaccc tgccttaaac 8580
aaacaacaaa gaaaggccaa gtcccataag tgggtcaccg cgccgagact ggggtccacg 8640
ggacacccca gccacgccaa gccgggaagt ccccgcctcc tggagctgaa cccgcccctc 8700
tcccagaggt ggagctgcgg ggggcgggaa caggcacgga gaaaataaac aagactaaaa 8760
agtcctgagt agcgctgtgt ggccgcaaac ctgaacccac cttttgcacc acgcgggacc 8820
cggcactctt cctgccaccc acccctgaga gggctgcgcg gccgacccca gtactagaaa 8880
acactcgtca cctcactcaa gacgggtacg aaggccaacg gacgccttcc tttagaacgc 8940
tcagcacaca gagcaacttc tcacgcctac tctcaaatgg cgtactccaa actagcactc 9000
ccgacgtcca gctgtgaacc cagagcggcg gaaagcccct gaacccagcg cccgggcatg 9060
cgcagacgcg ttgttgtggt gggcgtggct ccctccggac ccggcgcccc gccctccgcc 9120
ccgtgtccgc atgcgcgact gagccgggtg gatggtactg ctgcatccgg gtgtctggag 9180
gctgtggccg ttttgttttc ttggctaaaa tcgggggagt gaggcgggcc ggcgcggc   9238
```

<210> 21
<211> 1249
<212> DNA
<213> Homo sapiens

<400> 21

```
ctgatctaca taggaattgt tttcaagaca tttctgcatt cctctagtga cagggtgctc 60

actacctcat gagtatttca gtggacaact gtaatggtca ataaagtatc cactttccac 120
ctccctgcag ctcctggccc tggctttatt ctctggggct ccacacattc agtttacact 180
cagtggccag tggctgggac cattgtagaa aataaggaaa ctccaattcc ttccttcttt 240
tcttcctctt tcatctcttc ctccctctct acatccctct ctctcttcct tccttcctcg 300
acacttacca tgtaccagac cttctgccag gcacatggat gggagcacag gggaagttgg 360
ctgcagggtt agaactaagt cccaagcccc ctaaagctca tgccagggga ctggactgtc 420
cagtactgag ggatggggat gctgaggctg gtggccttcc tcaaatgcac tgtagtgccc 480
caggcagagt cctgggctgc cctgtgagga ggtgaccaga ggtagagcaa cttcacccta 540
aggctggatc aggatcccct ccaggttttt actagagcca aacccacatc tcctttctct 600
tctgccaccc cccttaaaa tgcttagaaa cacatagatt taaatacaaa ttcaaatgta 660
agtaatttca actgtgtaac tatgaggagt cagttctacg tgggtcctat ctgtatcctc 720
cccagggctc agctccattc tttgctttca ttcattctca ttcaatacat tgttgttaag 780
agctcactgg gtgccctctc tgtcatgtag taaggtttta aaaagaaagc ctcttctgag 840
cttcagtttc cttattcata aaataggagt attgatccgt tccttgcttt tcttacaagg 900
atatgctgaa gatgactgaa gtacagagta aagaaggatt atgtttgggt gtcaaaggaa 960
tagaatgccc tctttcaaac tgagcacagc aggaacctgt aacaggaaca cagcaacttg 1020
ttgaatgaat gacaatattg gaaaacatac atttcctccc ctccccatca tagtccctct 1080
gcttccgtgt taactccata gagaggccag cacaaccagc cttgcagcct gagataaggc 1140
ctttggcggg tgtctcccct atcgctccct caagccctca agtaggtgtt ggagagaggg 1200
gtgatgcctg gtgctggtgg aacccctgca cagagacgga cacaggatg            1249
```

<210> 22
<211> 699
<212> DNA
<213> Homo sapiens

<400> 22

```
ctagagaggg aagtttttga aaattaaaca ctgtctaatt ttctgcaaag tttttattca 60
tgaattaaga gtatttccct tagtccatta ttcccaaggc aaatatggaa gtttgatcat 120
atgctaatca tactaaagct ggattctctt taagagattg agaaattaaa aggcaaaagc 180
tgatatatca tgtttagtta tactgtgagt cttataagaa gctgggaggc aaccccatta 240
actcaccaga atacagaact cagtctcaca acttaaatat aattcctctc aaaccttttc 300
ctcaaagtta aattctgaaa ataatcttgt gattaagaga agaaggctgt ccaccaatgg 360
acttatctgt tatttcttcc ttattgtgag cttaatggca tgacaaagca gaggcaaaga 420
ggcatacatc aattcttcaa agtaggaagt caaaaaggtc agagcttcca cagcatggca 480
acagctttgc agatgcccac atcgtgatag ttgaaatagc aaagcccagc aaaggttaaa 540
gctgaaaatg ccaaaagccc tgccttggca gctttctgcg aggcatcccc atgaacatag 600
tcagtaacaa cttgtccaag gccccagtga ccatgaagag tgagggctgc agccagggaa 660
tagtccgtcg cagagcaagg attcaaataa gcagccgga                       699
```

<210> 23
<211> 699
<212> DNA
<213> Homo sapiens

<400> 23

```
ctagagaggg aagtttttga aaattaaaca ctgtctaatt ttctgcaaag tttttattca 60
tgaattaaga gtatttccct ttgtccatta ttcccaaggc aaatatggaa atttgatcat 120
gtactaatca tactaaagct ggattctctt taagagattg agaaattaaa aggcaaaagc 180
tgatatgtca tgtttagtta tattgtgagt cttataagaa gctgggaggc aaccccatta 240
actcaccaga atacagaact cagtctcaca acttaaatat aattcctctc aaaccttttc 300
ctcaaagtta aattctgaaa ataatcttgt gattaagaga agaaggctgt ccaccaatgg 360
acttatctgt tatttcttcc ttattgtgag cttaatggca tgacaaagca gaggcaaaga 420
ggcatacatc aattcttcaa agtaggaagt caaaaaggtc agagcttcca cagcatggca 480
acagctttgc agatgcccac atcgtgatag ttgaaatagc aaagcccagc aaaggttaaa 540
gctgaaaatg ccaaaagccc tgccttggca gctttctgcg aggcatcccc atgaacatag 600
tcagtaacaa cttgtccaag gccccagtga ccatgaagag tgagggctgc agccagggaa 660
tagtccgtcg cagagcaagg attcaaataa gcagccgga                        699
```

<210> 24
<211> 699
<212> DNA
<213> Homo sapiens

<400> 24

```
ctagagaggg aagtttttga aaattaaaca ctgtctaatt ttctgcaaag tttttattca 60
tgaattaaga gtatttccct ttgtccatta ttcccaaggc aaatatggaa atttgatcat 120
gtactaatca taataaagct ggattctctt taagagattg agaaattaaa aggcaaaagc 180
tgatatatca tgtttagtta tattgtgagt cttataagaa gctgggaggc aaccccatta 240
actcaccaga atacagaact cagtctcaca acttagatat aattcctctc aaaccttttc 300
ctcaaagatt aaattctgaa aataatcttg tgattaagag aagaaggctg tccaccaatg 360
ggcttatctg ttatttcttc cttattgtga gcttaatggc atgacaaagc agaggcaaag 420
aggcatacat caattcttca aagtaggaag tcaaaaaggt cagagcttcc acagcatggc 480
aacagctttg cagatgccca catcgtgata gttgaaatag caaagcccag caaaggttaa 540
agctgaaaat gccaaaagcc ctgccttggc agctttctgc gaggcatccc catgaacata 600
atcagtaaca acttgttcaa ggccccagtg accatgaaga gtgagggctg cagccgggaa 660
tagtccgtcg cagagcaagg attcaaataa gcagccgga                        699
```

<210> 25
<211> 994
<212> DNA
<213> Homo sapiens

<400> 25

```
ttgtatctct ttttacagct acctcccatt tcccttctat ttcaagctag taacacagtt 60
ttcttttaaa ttcatttatt taaatgtaaa aataagtcta tttggagaaa aaaaattttt 120
aatagcatct ctggaatgcc agtatggcta aattcatgaa tgttgtcctc aaatgctgaa 180
atctgggaag catctggcca agctttgtgg acaggcctgc ctagtttgaa tcccaagagc 240
cactcattcc gagccacaaa acattggaat tcttggttca cttccctaac ctgaacttgt 300
cctctgtgaa atagggacat taatagctca ctcacagggc tgctgtgagg acatgtgttg 360
agctgagggt ctcgccaggg gagaccctgt gcagggagac tgttatcatg gtgatggatt 420
tctgcttcat tcatttcttt ttccagacag catcatatag aatgagttgt ggggtggcag 480
tcagcaggtt tgggtttatc ctctattctg ccacttatta cttaaaaaaa aaaacccaac 540
ttatatagta taagctatat ccagaaaagt gcaaatatca tacaagtacc atttgatgaa 600
tcttctgata tccccacata accaacaccc agaacctctt cttgtctcat tccaggataa 660
ccactaacct gacttctaac agcatcagtc agttttgtct gtttttgtac attatatatg 720
tgatggtttg aatgtgtccc ccaaatttca tgtgctagaa acttaatcct tcaattcata 780
tgttgatgct atttggagga agggcctttg ggaagtaatt aggattagat aaggtcatgg 840
ggtgaggtat gatggcactg gtgacttata agaagagaaa gagaaatctg agctggcatg 900
ctcttgccct ctcaccgtgt gatgacttct ccatgtcatg atgcagcaag aaggccctca 960
ccagatggtg gcaccatgct tttggacttc ccag                             994
```

<210> 26
<211> 1000
<212> DNA
<213> Homo sapiens

<400> 26

```
ttgtatctct ttttacagct acctcccatt tcccttctat ttcaagctag taacacagtt 60
ttcttttaaa ttcatttatt taaatgtaaa aataagtcta tttggagaaa aaaaattttt 120
aatagcatct ctggaaggcc agtatggcta aattcatgaa tgttgtcctc aaatgctgaa 180
atctgggaag catctggcca agctttgtgg acaggcctgc ctagtttgaa tcccaagagc 240
cactcattcc gagccacaaa acattggaat tcttggttca cttccctaac ctgaacttgc 300
cctctgtgaa atagggacat taatagctca ctcacagggc tgctgtgagg acatgtgttg 360
agctgagggt ctggtcaggg gagaccctgt gcagggagac tgttatcatg gtgatggatt 420
tctgcttcat tcatttcttt ttccagacag catcatatag aatgagttgt ggggtggcag 480
tcagcaggtt tgggtttatc ctctattctg ccacttatta cttaaaaaaa ccccaaaaaa 540
cccaacttat atagtataag ctatatccag aaaagtgcaa atatcataca agtaccattt 600
gatgaatctt ctgatatccc cacataacca acacccagaa cctcttcttg tctcattcca 660
ggataaccac taacctgact tctaacagca tcagtcagtt ttgtctgttt ttgtacatta 720
tatatgtgat ggtttgaatg tgtcccccaa atttcatgtg ctggaaactt aatccttcaa 780
```

```
ttcatatgtt gatgctattt ggaggaaggg cctttgggaa gtaattagga ttagataagg 840
tcatggggtg aggtatgatg gcactggtga cttataagaa gagaaagaga aatctgagct 900
ggcatgctct tgccctctca ctgtgtgatg acttctccat gtcatgatgc agcaagaagg 960
ccctcaccag atggtggcac catgcttttg gacttcccag                        1000
```

<210> 27
<211> 999
<212> DNA
<213> Homo sapiens

<400> 27

```
ttgtatctct ttttacagct acctcccatt tcccttctat ttcaagctag taactcagtt 60
ttcttttaaa ttcaattatt taaatgtaaa aataagtcta tttggagaaa aaaaatttta 120
atagcatctc tggaatgcca gtatggctaa attcatgaat gttgtcctca aatgctgaaa 180
tctgggaagc atctggccaa gctttgtgga caggcctgcc tagtttgaat cccaagagcc 240
acccagtcca agccacaaaa cattggaatt cttggttcac ttccctaacc tgaacttgcc 300
ctctgtgaaa tagggacact aatagctcac tcacagggct gctgtgagga catgtgttga 360
gctgagggtc tcgccagggg agaccctgtg cagggagact gttatcatgg tgatggattt 420
ctgcttcatt catttctttt tccagacagc atcatataga atgagttgtg gggtggcagt 480
cagcaggttt gggtttatcc tctattctgc cacttattac ttaaaaaaac cccaaaaaac 540
ccaacttata tagtataagc tatatccaga aaagtgcaaa tatcatacaa gtaccatttg 600
atgaatcttc tgatatcccc acataaccaa cacccagaac ctcttcttgt ctcattccag 660
gataaccact aacctgactt ctaacagcat cagtcagttt tgtctgtttt tgtacattat 720
atatgtgatg gtttgaatgt gtcccccaaa tttcatgtgc tggaaactta atccttcaat 780
tcatatgttg atggtttttg gaggaagggc ctttgggaag taattaggat tagataaggt 840
catggggtga ggtatgatgg cactggtgac ttataagaag agaaagagaa atctgagctg 900
gcatgctctt gccctctcac tgtgtgatga cttctccatg tcatgatgca gcaagaaggc 960
cctcaccaga tggtggcacc atgcttttgg acttcccag                        999
```

<210> 28
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 28
ggctaaatat tttgatgacc aagtt 25

<210> 29
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 29
gcagccaact tcccctgtg 19

<210> 30
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 30
gctctacctt ggtcacctcc 20

<210> 31
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 31
aggtcacatc catttatccc actg 24

<210> 32
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 32
agaagatggg ggaatctttt tcct 24

<210> 33
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 33
ttgtgactgg gctagaaaga aggtg 25

<210> 34
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 34
tgttgcctgc atttgtacgt gag 23

<210> 35
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 35
ttctgtctgg gttggggagg g 21

<210> 36
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 36
ggaggggtta atatgggtgg c 21

<210> 37
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 37
tttgtcctgg ttgcctgtgg tc 22

<210> 38
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 38
caaatcctgt tgactggtct cgg 23

<210> 39
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 39
aacggctcca tcacccctaa ag 22

<210> 40
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 40
cccactccta gataccaacc caag 24

<210> 41
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 41
ctttatgcac tgcctcgttg aatc 24

<210> 42
<211> 22
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

5

<400> 42
ttgactggtg tggttgctgt tg 22

<210> 43
10 <211> 22
<212> DNA
<213> Artificial Sequence

<220>
15 <223> Description of Artificial Sequence: artificial primer

<400> 43
gcagaaaggg gagttgatgc tg 22

20 <210> 44
<211> 23
<212> DNA
<213> Artificial Sequence

25 <220>
<223> Description of Artificial Sequence: artificial primer

<400> 44
ctgacaaagt tgagagccca ctg 23

30

<210> 45
<211> 24
<212> DNA
<213> Artificial Sequence

35

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 45
40 ttaagcctac atccacatgc tgag 24

<210> 46
<211> 22
<212> DNA
45 <213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

50 <400> 46
ccttggtctg ccagaatttt ca 22

<210> 47
<211> 24
55 <212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificial primer

<400> 47
gtttggcatc ataggagatt tggc 24

<210> 48
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 48
cctgtcccca tgattcagtt acc 23

<210> 49
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 49
acgtacaaat gcaggcaac 19

<210> 50
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 50
cctttttttg tttgtttttg gcggtgc 27

<210> 51
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 51
agcttactgg atgaccacca 20

<210> 52
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 52

gactgggggg aaaagcgcaa tac 23

<210> 53
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 53
gtattgcgct tttcccccca gtc 23

<210> 54
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 54
tgacttgctc tcatcccaca tg 22

<210> 55
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 55
gggcttgaag caagtaaatg gaag 24

<210> 56
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 56
gctatcaata ttttcttggt tacagacac 29

<210> 57
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 57
gttcactgcc ataagtcttc agtgc 25

<210> 58

<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 58
tggccgcact gaagacttat gg 22

<210> 59
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 59
cagctgcatc tatgataatc cacc 24

<210> 60
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 60
atggacaagt ccgaggtgat ag 22

<210> 61
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 61
atcacctcgg acttgtccat tc 22

<210> 62
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 62
gcaatcagag atccaaaggc caac 24

<210> 63
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 63
gttggccttt ggatctctga ttgc 24

<210> 64
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 64
gacatagtat accctggaat tgctgt 26

<210> 65
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 65
acagcaattc cagggtatac tatgtc 26

<210> 66
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial primer

<400> 66
ctcccccgat tttagccaag aa 22
1
17

## Claims

1. A nucleic acid molecule that is an *RHD* gene comprising a single nucleotide substitution within a 5' or 3' splice site, wherein said substitution gives rise to a mutation within a 4-nucleotide sequence, a 6-nucleotide sequence or an 8-nucleotide sequence comprising the consensus splice site at the exon9/intron9 boundary or at the exon3/intron3 boundary and wherein said nucleic acid molecule correlates with a Del-phenotype.

2. The nucleic acid molecule of claim 1 wherein said substitution gives rise to an *RHD*(K409K) mutation.

3. The nucleic acid molecule of claim 2 wherein said substitution is a substitution at the 5' splice site intron 9 from AGgt to AAgt.

4. The nucleic acid molecule of claim 1 wherein said substitution gives rise to an *RHD*(G468+1)A) mutation.

5. The nucleic acid molecule of claim 4 wherein said substitution is a substitution at the 5' splice site intron 3 from ACgt to ACat.

6. A nucleic acid molecule that is an *RHD* gene comprising a single nucleotide substitution within the coding region of the *RHD* gene or within a 5' or 3' splice site wherein said nucleotide substitution gives rise to a stop codon at codon 16, to a stop codon at codon 330, to a missense mutation at codon 212 or to a mutation within a 4-nucleotide sequence, a 6-nucleotide sequence or an 8-nucleotide sequence comprising the consensus splice site at the exon8/intron8 boundary wherein said nucleic acid molecule correlates with an *RHD* negative phenotype.

7. The nucleic acid molecule of claim 6 wherein said substitution gives rise to an *RHD*(W16X) mutation.

8. The nucleic acid molecule of claim 7 wherein said substitution is a G → A substitution at nucleotide position 48.

9. The nucleic acid molecule of claim 6 wherein said substitution gives rise to an RHD(Y330X) mutation.

10. The nucleic acid molecule of claim 9 wherein said substitution is a C → G substitution at nucleotide position 985.

11. The nucleic acid molecule of claim 6 wherein said substitution gives rise to an *RHD*(G212V) missense mutation.

12. The nucleic acid molecule of claim 11 wherein said substitution is a G → T substitution at nucleotide position 635.

13. The nucleic acid molecule of claim 6 wherein said substitution gives rise to an *RHD*(G1153(+1)A) mutation.

14. The nucleic acid molecule of claim 13 wherein said substitution is a substitution at the 5' splice site intron 8 from AGgt to AGat.

15. A protein product of the *RHD* gene encoded by the nucleic acid molecule of any one of claims 1 to 5.

16. An oligonucleotide comprising 12 to 50 nucleotides hybridizing under stringent conditions to a portion of the nucleic acid molecule of any one of claims 1 to 14 wherein said portion comprises said (missense) mutation or said stop codon or to the complementary portion thereof.

17. An antibody or aptamer or phage specifically binding to a protein product of the *RHD* gene of claim 15.

18. A method for testing for the presence of a nucleic acid molecule encoding a mutant Rhesus D antigen as **characterized by** the nucleic acid molecule of any one of claims 1 to 14 in a sample comprising hybridizing the oligonucleotide of claim 16 under stringent conditions to nucleic acid molecules comprised in the sample obtained from a human and detecting said hybridization.

19. The method of claim 18 further comprising digesting the product of said hybridization with a restriction endonuclease and analyzing the product of said digestion.

20. A method for testing for the presence of a nucleic acid molecule encoding a mutant Rhesus D antigen as **characterized by** the nucleic acid molecule of any one of claims 1 to 14 in a sample comprising determining the nucleic acid sequence of at least a portion of the nucleic acid molecular structure or nucleic acid molecule of any one of claims 1 to 14, said portion encoding said (missense) mutation or said stop codon.

21. The method of claim 20 further comprising, prior to determining said nucleic acid sequence, amplification of at least said portion of said nucleic acid molecule or structure.

22. A method for testing for the presence of a nucleic acid molecule encoding a mutant Rhesus D antigen as **characterized by** the nucleic acid molecule of any one of claims 1 to 14 in a sample comprising carrying out an amplification reaction using a set of primers that amplifies at least a portion of said sequence
wherein at least one of the primers employed in said amplification reaction is the oligonucleotide of claim 16.

23. The method of claim 21 to 22 wherein said amplification is effected by or said amplification reaction is the polymerase chain reaction (PCR).

24. The method of claim 23 wherein said PCR is PCR-RFLP, PCR-SSP or long-range PCR.

25. The method of any one of claims 21 to 24 wherein the molecular weight of the amplification product is analyzed.

**26.** A method for testing for the presence of the nucleic acid molecule of any one of claims 1 to 14 encoding *RHD* positive alleles comprising the following steps:

(a) isolating the DNA from a blood sample or blood donor;
(b) hybridizing at least two oppositely oriented primers under stringent conditions to the DNA so as to carry out a PCR;
(c) amplifying the target sequence;
(d) separating the amplification products on a gel; and
(e) analyzing the amplicons

wherein said *RHD* positive alleles are derived from a serologically RhD negative sample.

**27.** The method of claim 26 wherein said sample is selected from a Caucasian population.

**28.** A method for testing for the presence of a protein product of the *RHD* gene of claims 15 in a sample comprising assaying a sample obtained from a human for specific binding to the antibody or aptamer of phage of claim 17.

**29.** A method for testing for the presence of a protein product of the *RHD* gene encoding the nucleic acid molecule of any one of claims 1 to 5 in a sample comprising utilizing direct agglutination methods, indirect antiglobulin tests, monoclonal anti-D antibodies and/or adsorption/elution techniques.

**30.** The method of any one of claims 18 to 29 wherein said sample is blood, serum, plasma, fetal tissue, saliva, urine, mucosal tissue, mucus, vaginal tissue, skin, hair, hair follicle or another human tissue.

**31.** The method of claim 30 comprising enrichment of fetal cells or extraction of fetal DNA or mRNA from maternal tissue, like peripheral blood, serum or plasma.

**32.** The method of any one of claims 18 to 31 wherein said nucleic acid molecule or proteinaceous material from said sample is fixed to a solid support.

**33.** The method of claim 32 wherein said solid support is a chip.

**34.** Use of the nucleic acid molecule of any one of claims 1 to 14 for the analysis of a negative or a positive Rhesus D phenotype.

**35.** Use of the nucleic acid molecule of any one of claims 1 to 14, or the protein product of the *RHD* gene of claim 15 for the assessment of the affinity, avidity and/or reactivity of monoclonal antibodies or of polyclonal antisera preferably anti-D antisera, anti-globulin or anti-human-globulin antisera.

**36.** Use of cells, preferably red blood cells from probands carrying the nucleic acid molecule of any one of claims 1 to 14 for the assessment of the affinity, avidity and/or reactivity of monoclonal anti-D antibodies or of polyclonal anti-D or of anti-globulin or of anti-human-globulin antisera or of preparations thereof.

**37.** A method for the characterization of the monoclonal antibodies or polyclonal antisera or of a preparation thereof said method comprising

(a) testing the nucleic acid molecule of a sample of a proband for the presence of a mutation as defined in any one of claims 1 to 14;
(b) correlating, on the basis of the mutation and the allelic status of the *RHD* gene, the nucleic acid with the density of the protein product of the *RHD* gene on the surface of red blood cells of said proband;
(c) reacting said monoclonal antibodies or polyclonal antisera or said preparation thereof with a cell carrying the protein product of the *RHD* gene on its surface;
(d) characterizing said monoclonal antibodies or polyclonal antisera or said preparation thereof on the basis of the results obtained in step (c).

**38.** The method of claim 37 wherein said characterization comprises the determination of reactivity, sensitivity, avidity, affinity specificity and/or other characteristics of antibodies and antisera.

39. The method of claim 37 or 38 wherein said cell carrying the protein product of the *RHD* gene on its surface is a red blood cell.

40. A method for determining whether a patient in need of a blood transfusion is to be transfused with RhD negative blood from a donor comprising the step of testing a sample from said patient for the presence of one or more nucleic acid molecules of any one of claims 1 to 14 wherein a positive testing for two different of said nucleic acid molecules is indicative of the need for a transfusion with Rh negative blood.

41. A method of assessing of the risk of the RhD negative mother of conceiving or carrying an RhD positive fetus or of the risk of a mother having an anti-D titer of conceiving or carrying a fetus at risk to develop haemolytic disease of the newborn comprising assessing a sample obtained from the father of the fetus for the presence of one or more nucleic acid molecules as defined in any one of claims 1 to 14.

42. A preparation comprising the antibody or aptamer or phage of claim 17.

43. A method of identifying an antibody $V_H$ or $V_L$ chain or a combination thereof or an aptamer specifically binding to a protein product of the *RHD* gene of claim 15 comprising

(a) contacting the protein product of the *RHD* gene of claim 15 with a phage library displaying $V_H$ or $V_L$ chains or combinations thereof on the surface of the phage or with aptamers;
(b) identifying phage or aptamers that bind to said protein product; and optionally
(c) repeating steps (a) and (b) one or more times.

44. A method of identifying a monoclonal antibody specifically binding to a protein product of the *RHD* gene of claim 15 comprising

(a) contacting the protein product of claim 15 with one or more monoclonal antibodies;
(b) identifying monoclonal antibodies that bind to said protein; and optionally
(c) repeating steps (a) and (b) one or more times.

45. A method of identifying an antibody $V_H$ or $V_L$ chain or a combination thereof or an aptamer specifically binding to a protein product of the *RHD* gene of claim 15 comprising

(aa) contacting said protein product; and
(ab) a normal D polypeptide
wherein the normal D polypeptide is present in a molar mass that is higher, equal or less than the protein product of the *RHD* gene of (aa) with a phage library displaying $V_H$ or $V_L$ chains or combinations thereof on the surface of the phage or with aptamers;
(b) identifying phage or aptamers that bind to said protein product of the *RHD* gene of (aa); and optionally
(c) repeating steps (a) and (b) one or more times.

46. A method of identifying a monoclonal antibody specifically binding to a protein product of the *RHD* gene of claim 15 comprising

(aa) contacting the protein product of the *RHD* gene; and
(ab) a normal D polypeptide
wherein the normal D polypeptide is present in a molar mass which is higher, equal or less than the protein product of the *RHD* gene of (aa) with one or more monoclonal antibodies;
(b) identifying monoclonal antibodies that bind to said protein product of the *RHD* gene of (aa); and optionally
(c) repeating steps (a) and (b) one or more times.

47. The method of any one of claims 43 to 46 wherein the protein product of the *RHD* gene is exposed on the surface of a cell.

48. The method of any one of claims 43 to 47 wherein the polypeptide or host cell is affixed to a solid support.

49. The method of any one of claims 43 to 48 wherein subsequent to step (b) or (c) the following step is carried out:

(d) identifying the amino acid sequence of the $V_H$ or $V_L$ chains and/or identifying the nucleic acid sequence encoding said amino acid sequence.

**50.** The method of any one of claims 43 to 46 wherein, in the case that only one round of selection is employed for the identification, the number of protein molecules of the *RHD* gene of (a) is in molar excess over the number of phage particles.

**51.** Use of cells, preferably red blood cells comprising the protein product of the *RHD* gene of claim 15, from probands for the assessment of the affinity, avidity and/or reactivity of monoclonal anti-D of claim 17 or of polyclonal anti-D or of anti-globulin or of anti-human-globulin antisera or of preparations thereof.

**52.** Kit comprising

(a) the oligonucleotide of claim 16, and/or
(b) the antibody of claim 17; and/or
(c) the aptamer of claim 17, and/or
(d) the phage of claim 17; and/or
(e) a pair of primers useful for carrying out the amplification reaction of any one of claims 20 and 25.

**Patentansprüche**

**1.** Nukleinsäuremolekül, das ein *RHD*-Gen ist, das einen Einzelnukleotid-Austausch in einer 5-’ oder 3’-Spleiß-Stelle umfasst, wobei der Austausch eine Mutation in einer Sequenz von 4 Nukleotiden, einer Sequenz von 6 Nukleotiden oder einer Sequenz von 8 Nukleotiden hervorruft, die die Konsensus-Spleiß-Stelle an der Exon9/Intron9-Grenze oder an der Exon3/Intron3-Grenze umfasst und wobei das Nukleinsäuremolekül mit einem Del-Phänotyp korreliert.

**2.** Nukleinsäuremolekül nach Anspruch 1, wobei der Austausch eine *RHD*(K409K)-Mutation hervorruft.

**3.** Nukleinsäuremolekül nach Anspruch 2, wobei der Austausch ein Austausch von AGgt zu AAgt an der 5’-Spleiß-Stelle von Intron 9 ist.

**4.** Nukleinsäuremolekül nach Anspruch 1, wobei der Austausch eine *RHD*(G468+1)A)-Mutation hervorruft.

**5.** Nukleinsäuremolekül nach Anspruch 4, wobei der Austausch ein Austausch von ACgt zu ACat an der 5’-Spleiß-Stelle von Intron 3 ist.

**6.** Nukleinsäuremolekül, das ein *RHD*-Gen ist, das einen Einzelnukleotid-Austausch im kodierenden Bereich des *RHD*-Gens oder in einer 5-’ oder 3’-Spleiß-Stelle umfasst, wobei der Nukleotid-Austausch ein Stoppkodon in Kodon 16, ein Stoppkodon in Kodon 330, eine Missense-Mutation in Kodon 212 oder eine Mutation in einer Sequenz von 4 Nukleotiden, einer Sequenz von 6 Nukleotiden oder einer Sequenz von 8 Nukleotiden hervorruft, die die Konsensus-Spleiß-Stelle an der Exon8/Intron8-Grenze umfasst und wobei das Nukleinsäuremolekül mit einem *RHD*-negativen Phänotyp korreliert.

**7.** Nukleinsäuremolekül nach Anspruch 6, wobei der Austausch eine *RHD*(W16X)-Mutation hervorruft.

**8.** Nukleinsäuremolekül nach Anspruch 7, wobei der Austausch ein G $\rightarrow$ A Austausch in Nukleotidposition 48 ist.

**9.** Nukleinsäuremolekül nach Anspruch 6, wobei der Austausch eine *RHD*(Y330X)-Mutation hervorruft.

**10.** Nukleinsäuremolekül nach Anspruch 9, wobei der Austausch ein C $\rightarrow$ G Austausch in Nukleotidposition 985 ist.

**11.** Nukleinsäuremolekül nach Anspruch 6, wobei der Austausch eine *RHD*(G212V)-Missense-Mutation hervorruft.

**12.** Nukleinsäuremolekül nach Anspruch 11, wobei der Austausch ein G $\rightarrow$ T Austausch in Nukleotidposition 635 ist.

**13.** Nukleinsäuremolekül nach Anspruch 6, wobei der Austausch eine *RHD*(G1153(+1)A)-Mutation hervorruft.

**14.** Nukleinsäuremolekül nach Anspruch 13, wobei der Austausch ein Austausch von AGgt zu AGat an der 5'-Spleiß-Stelle von Intron 8 ist.

**15.** Proteinprodukt des *RHD*-Gens, das von dem Nukleinsäuremolekül nach einem der Ansprüche 1 bis 5 kodiert wird.

5

**16.** Oligonukleotid umfassend 12 bis 50 Nukleotide, die unter stringenten Bedingungen an einen Teil des Nukleinsäuremoleküls nach einem der Ansprüche 1 bis 14 hybridisieren, wobei der Teil die (Missense-) Mutation oder das Stoppkodon oder den dazu komplementären Teil umfasst.

10

**17.** Antikörper oder Aptamer oder Phage, der spezifisch an ein Proteinprodukt des *RHD*-Gens nach Anspruch 15 bindet.

**18.** Verfahren zum Testen auf das Vorhandensein eines Nukleinsäuremoleküls, das ein mutiertes Rhesus D-Antigen, wie durch das Nukleinsäuremolekül nach einem der Ansprüche 1 bis 14 **gekennzeichnet** kodiert, in einer Probe, umfassend das Hybridisieren des Oligonukleotids nach Anspruch 16 unter stringenten Bedingungen an Nukleinsäuremoleküle, die in der von einem Menschen erhaltenen Probe enthalten sind, und den Nachweis der Hybridisierung.

15

**19.** Verfahren nach Anspruch 18, des weiteren umfassend das Verdauen des Hybridisierungsprodukts mit einer Restriktionsendonuklease und das Analysieren des Verdauungsprodukts.

20

**20.** Verfahren zum Testen auf das Vorhandensein eines Nukleinsäuremoleküls, das ein mutiertes Rhesus D-Antigen, wie durch das Nukleinsäuremolekül nach einem der Ansprüche 1 bis 14 **gekennzeichnet** kodiert, in einer Probe, umfassend das Bestimmen der Nukleinsäuresequenz von mindestens einem Teil der Nukleinsäuremolekülstruktur oder des Nukleinsäuremoleküls nach einem der Ansprüche 1 bis 14, wobei der Teil die (Missense-) Mutation oder das Stoppkodon kodiert.

25

**21.** Verfahren nach Anspruch 20, des weiteren umfassend die Amplifikation von mindestens einem Teil des Nukleinsäuremoleküls oder der Nukleinsäuremolekülstruktur vor der Bestimmung der Nukleinsäuresequenz.

30

**22.** Verfahren zum Testen auf das Vorhandensein eines Nukleinsäuremoleküls, das ein mutiertes Rhesus D-Antigen, wie durch das Nukleinsäuremolekül nach einem der Ansprüche 1 bis 14 **gekennzeichnet** kodiert, in einer Probe, umfassend das Ausführen einer Amplifikationsreaktion unter Verwendung eines Primersatzes, der mindestens einen Teil der Sequenz amplifiziert, wobei mindestens einer der in der Amplifikationsreaktion verwendeten Primer das Oligonukleotid nach Anspruch 16 ist.

35

**23.** Verfahren nach Anspruch 21 oder 22, wobei die Amplifikation mittels der Polymerase-Kettenreaktion (PCR) erfolgt oder die Polymerase-Kettenreaktion (PCR) ist.

**24.** Verfahren nach Anspruch 23, wobei die PCR eine PCR-RFLP, eine PCR-SSP oder eine Long-Range-PCR ist.

40

**25.** Verfahren nach einem der Ansprüche 21 bis 24, wobei das Molekulargewicht des Amplifikationsprodukts analysiert wird.

**26.** Verfahren zum Testen auf das Vorhandensein des Nukleinsäuremoleküls nach einem der Ansprüche 1 bis 14, das *RHD*-positive Allele kodiert, umfassend die folgenden Schritte:

45

(a) Isolieren der DNA aus einer Blutprobe oder aus einer Probe eines Blutspenders;
(b) Hybridisieren von mindestens zwei entgegengesetzt ausgerichteten Primern unter stringenten Bedingungen an die DNA zum Ausführen einer PCR;
(c) Amplifizieren der Zielsequenz;
(d) Auftrennen der Amplifikationsprodukte in einem Gel; und
(e) Analysieren der Amplikons

50

wobei die *RHD*-positiven Allele aus einer serologisch RhD-negativen Probe stammen.

55

**27.** Verfahren nach Anspruch 26, wobei die Probe aus einer kaukasischen Population ausgewählt ist.

**28.** Verfahren zum Testen auf das Vorhandensein eines Proteinprodukts des *RHD*-Gens nach Anspruch 15 in einer

Probe, umfassend das Untersuchen einer von einem Menschen erhaltenen Probe auf spezifische Bindung des Antikörpers oder Aptamers oder Phagen nach Anspruch 17.

**29.** Verfahren zum Testen auf das Vorhandensein eines Proteinprodukts des *RHD*-Gens, das das Nukleinsäuremolekül nach einem der Ansprüche 1 bis 5 kodiert, in einer Probe, umfassend die Verwendung von direkten Agglutinierungsverfahren, indirekten Antiglobulin-Tests, monoklonalen anti-D-Antikörpern und/oder Adsorptions-/Elutionstechniken.

**30.** Verfahren nach einem der Ansprüche 18 bis 29, wobei die Probe Blut, Serum, Plasma, fötales Gewebe, Speichel, Urin, Schleimhautgewebe, Schleim, vaginales Gewebe, Haut, Haar, Haarfollikel oder anderes menschliches Gewebe ist.

**31.** Verfahren nach Anspruch 30, umfassend das Anreichern fötaler Zellen oder die Extraktion fötaler DNA oder mRNA aus mütterlichem Gewebe wie peripherem Blut, Serum oder Plasma.

**32.** Verfahren nach einem der Ansprüche 18 bis 31, wobei das Nukleinsäuremolekül oder das proteinöse Material der Probe an einen festen Träger gebunden ist.

**33.** Verfahren nach Anspruch 32, wobei der feste Träger ein Chip ist.

**34.** Verwendung des Nukleinsäuremoleküls nach einem der Ansprüche 1 bis 14 für die Analyse eines negativen oder positiven Rhesus D-Phänotyps.

**35.** Verwendung des Nukleinsäuremoleküls nach einem der Ansprüche 1 bis 14 oder des Proteinpodukts des *RHD*-Gens nach Anspruch 15 für die Bestimmung der Affinität, der Avidität und/oder Reaktivität von monoklonalen Antikörpern oder von polyklonalen Antiseren, vorzugsweise anti-D-Antiseren, Antiglobulin- oder Antihumanglobulin-Antiseren.

**36.** Verwendung von Zellen, vorzugsweise roten Blutkörperchen von Probanden, die das Nukleinsäuremolekül nach einem der Ansprüche 1 bis 14 tragen, zur Bestimmung der Affinität, der Avidität und/oder Reaktivität von monoklonalen anti-D-Antikörpern oder von polyklonalem anti-D- oder von Antiglobulin- oder von Antihumanglobulin-Antiseren oder Zubereitungen davon.

**37.** Verfahren zur Charakterisierung von monoklonalen Antikörpern oder polyklonalen Antiseren oder einer Zubereitung davon, umfassend

(a) Testen des Nukleinsäuremoleküls einer Probe eines Probanden auf das Vorhandensein einer Mutation, wie in einem der Ansprüche 1 bis 14 definiert;
(b) Korrelieren der Nukleinsäure mit der Dichte des Proteinprodukts des *RHD*-Gens auf der Oberfläche von roten Blutkörperchen des Probanden auf der Grundlage der Mutation und des Allel-Status des *RHD*-Gens;
(c) Durchführen einer Reaktion der monoklonalen Antikörper oder der polyklonalen Antiseren oder der Zubereitung davon mit einer Zelle, die das Proteinprodukt des *RHD*-Gens auf ihrer Oberfläche trägt;
(d) Charakterisieren der monoklonalen Antikörper oder der polyklonalen Antiseren oder der Zubereitung davon auf der Grundlage der in Schritt (c) erhaltenen Ergebnisse.

**38.** Verfahren nach Anspruch 37, wobei die Charakterisierung die Bestimmung der Reaktivität, der Sensitivität, der Avidität, der Spezifität der Affinität und/oder anderer Eigenschaften von Antikörpern und Antiseren umfasst.

**39.** Verfahren nach Anspruch 37 oder 38, wobei die Zelle, die das Proteinprodukt des *RHD*-Gens auf ihrer Oberfläche trägt, ein rotes Blutkörperchen ist.

**40.** Verfahren zur Bestimmung, ob ein Patient, der eine Bluttransfusion benötigt, mit RhD-negativem Blut eines Spenders transfundiert werden soll, umfassend den Schritt des Testens auf das Vorhandensein von einem oder mehreren Nukleinsäuremolekülen nach einem der Ansprüche 1 bis 14 in einer Probe des Patienten, wobei ein positiver Test für zwei verschiedene dieser Nukleinsäuremoleküle anzeigt, dass eine Transfusion mit Rh-negativem Blut notwendig ist.

**41.** Verfahren zur Einschätzung des Risikos einer RhD-negativen Mutter, einen RhD-positiven Fötus zu empfangen oder zu tragen, oder des Risikos einer Mutter, die einen anti-D-Titer aufweist, einen Fötus zu empfangen oder zu

tragen, welcher ein Risiko trägt, eine hämolytische Erkrankung von Neugeborenen zu entwickeln, umfassend das Untersuchen einer Probe des Vaters des Fötus auf das Vorhandensein eines oder mehrer Nukleinsäuremoleküle, wie in einem der Ansprüche 1 bis 14 definiert.

5

**42.** Zubereitung umfassend den Antikörper oder das Aptamer oder den Phagen nach Anspruch 17.

**43.** Verfahren zur Identifizierung einer $V_H$- oder $V_L$-Kette eines Antikörpers oder einer Kombination davon oder eines Aptamers, die/das spezifisch an ein Proteinprodukt des *RHD*-Gens nach Anspruch 15 bindet, umfassend

10

(a) Inkontaktbringen des Proteinprodukts des *RHD*-Gens nach Anspruch 15 mit einer Phagenbibliothek, die $V_H$- oder $V_L$-Ketten oder Kombinationen davon auf der Oberfläche der Phagen präsentiert, oder mit Aptameren;
(b) Identifizieren der Phagen oder der Aptamere, die an das Proteinprodukt binden; und wahlweise
(c) Einmaliges oder mehrmaliges Wiederholen der Schritte (a) und (b).

15

**44.** Verfahren zur Identifizierung eines monoklonalen Antikörpers, der spezifisch an ein Proteinprodukt des *RHD*-Gens nach Anspruch 15 bindet, umfassend

(a) Inkontaktbringen des Proteinprodukts nach Anspruch 15 mit einem oder mehreren monoklonalen Antikörpern;
20
(b) Identifizieren monoklonaler Antikörper, die an das Protein binden; und wahlweise
(c) Einmaliges oder mehrmaliges Wiederholen der Schritte (a) und (b).

**45.** Verfahren zur Identifizierung einer $V_H$- oder $V_L$-Kette eines Antikörpers oder einer Kombination davon oder eines Aptamers, die/das spezifisch an ein Proteinprodukt des *RHD*-Gens nach Anspruch 15 bindet, umfassend

25

(aa) Inkontaktbringen des Proteinprodukts; und
(ab) eines normalen D-Polypeptids,
wobei das normale D-Polypeptid in einer molaren Masse vorhanden ist, die höher, gleich oder niedriger ist als die des Proteinprodukts des *RHD*-Gens gemäß (aa), mit einer Phagenbibliothek, die $V_H$- oder $V_L$-Ketten oder
30
Kombinationen davon auf der Oberfläche der Phagen präsentiert, oder mit Aptameren;
(b) Identifizieren der Phagen oder der Aptamere, die an das Proteinprodukt gemäß (aa) binden; und wahlweise
(c) Einmaliges oder mehrmaliges Wiederholen der Schritte (a) und (b).

**46.** Verfahren zur Identifizierung eines monoklonalen Antikörpers, der spezifisch an ein Proteinprodukt des *RHD*-Gens
35
nach Anspruch 15 bindet, umfassend

(aa) Inkontaktbringen des Proteinprodukts des *RHD*-Gens; und
(ab) eines normalen D-Polypeptids,
wobei das normale D-Polypeptid in einer molaren Masse vorhanden ist, die höher, gleich oder niedriger ist als
40
die des Proteinprodukts des *RHD*-Gens gemäß (aa), mit einem oder mehreren monoklonalen Antikörpern;
(b) Identifizieren monoklonaler Antikörper, die an das Proteinprodukt des *RHD*-Gens gemäß (aa) binden; und wahlweise
(c) Einmaliges oder mehrmaliges Wiederholen der Schritte (a) und (b).

45

**47.** Verfahren nach einem der Ansprüche 43 bis 46, wobei das Proteinprodukt des *RHD*-Gens auf der Oberfläche einer Zelle exponiert ist.

**48.** Verfahren nach einem der Ansprüche 43 bis 47, wobei das Polypeptid oder die Wirtszelle an einen festen Träger gebunden ist.

50

**49.** Verfahren nach einem der Ansprüche 43 bis 48, wobei nach Schritt (b) oder (c) der folgende Schritt durchgeführt wird:

(d) Identifizieren der Aminosäuresequenz der $V_H$- oder $V_L$-Ketten und/oder Identifizieren der Nukleinsäuresequenz, die die Aminosäuresequenz kodiert.

55

**50.** Verfahren nach einem der Ansprüche 43 bis 46, wobei für den Fall, dass nur eine Selektionsrunde für die Identifizierung durchgeführt wird, die Anzahl der Protein-Moleküle des *RHD*-Gens gemäß (a) in molarem Überschuss zu der Anzahl der Phagenpartikel ist.

**51.** Verwendung von Zellen, vorzugsweise roten Blutkörperchen, die das Proteinprodukt des *RHD*-Gens nach Anspruch 15 umfassen, aus Probanden zur Bestimmung der Affinität, der Avidität und/oder der Reaktivität von monoklonalem anti-D nach Anspruch 17 oder von polyklonalem anti-D oder Antiglobulin- oder Antihumanglobulin-Antiseren oder Zubereitungen davon.

**52.** Kit umfassend

(a) das Oligonukleotid nach Anspruch 16, und/oder
(b) den Antikörper nach Anspruch 17; und/oder
(c) den Aptamer nach Anspruch 17; und/oder
(d) den Phagen nach Anspruch 17; und/oder
(e) ein Primerpaar, das für die Amplifikationsreaktion nach einem der Ansprüche 20 und 25 einsetzbar ist.

## Revendications

**1.** Molécule d'acide nucléique qui est un gène *RHD* comprenant une substitution d'un seul nucléotide au niveau d'un site d'épissage 5' ou 3', dans laquelle ladite substitution donne naissance à une mutation dans une séquence de 4 nucléotides, une séquence de 6 nucléotides ou une séquence de 8 nucléotides comprenant le site d'épissage consensus à la limite exon9/intron9 ou à la limite exon3/intron3 et dans laquelle ladite molécule d'acide nucléique correspond à un phénotype Del.

**2.** Molécule d'acide nucléique selon la revendication 1, dans laquelle ladite substitution donne naissance à une mutation *RHD*(K409K).

**3.** Molécule d'acide nucléique selon la revendication 2, dans laquelle ladite substitution est une substitution au niveau du site d'épissage 5' de l'intron 9 de AGgt à AAgt.

**4.** Molécule d'acide nucléique selon la revendication 1, dans laquelle ladite substitution donne naissance à une mutation RHD(G468+1)A.

**5.** Molécule d'acide nucléique selon la revendication 4, dans laquelle ladite substitution est une substitution au niveau du site d'épissage 5' de l'intron 3 de ACgt à ACat.

**6.** Molécule d'acide nucléique qui est un gène *RHD* comprenant une substitution d'un seul nucléotide dans la région codante du gène *RHD* ou au niveau d'un site d'épissage 5' ou 3', dans laquelle ladite substitution de nucléotide donne naissance à un codon stop au codon 16, à un codon stop au codon 330, à une nutation faux-sens au codon 212 ou à une mutation dans une séquence de 4 nucléotides, une séquence de 6 nucléotides ou une séquence de 8 nucléotides comprenant le site d'épissage consensus à la limite exon8/intron8, dans laquelle ladite molécule d'acide nucléique correspond à un phénotype *RHD* négatif.

**7.** Molécule d'acide nucléique selon la revendication 6, dans laquelle ladite substitution donne naissance à une mutation *RHD*(W16X).

**8.** Molécule d'acide nucléique selon la revendication 7, dans laquelle ladite substitution est une substitution G $\rightarrow$ A au niveau du nucléotide 48.

**9.** Molécule d'acide nucléique selon la revendication 6, dans laquelle ladite substitution donne naissance à une mutation *RHD*(Y330X).

**10.** Molécule d'acide nucléique selon la revendication 9, dans laquelle ladite substitution est une substitution C $\rightarrow$ G au niveau du nucléotide 985.

**11.** Molécule d'acide nucléique selon la revendication 6, dans laquelle ladite substitution donne naissance à une mutation faux-sens *RHD*(G212V).

**12.** Molécule d'acide nucléique selon la revendication 11, dans laquelle ladite substitution est une substitution G $\rightarrow$ T au niveau du nucléotide 635.

**13.** Molécule d'acide nucléique selon la revendication 6, dans laquelle ladite substitution donne naissance à une mutation *RHD*(G1153(+1)A).

**14.** Molécule d'acide nucléique selon la revendication 13, dans laquelle ladite substitution est une substitution au niveau du site d'épissage 5' de l'intron 8 de AGgt à AGat.

**15.** Produit protéique du gène *RHD* codé par la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 5.

**16.** Oligonucléotide comprenant de 12 à 50 nucléotides s'hybridant dans des conditions stringentes à une partie de la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 14, dans lequel ladite partie comprend ladite mutation (faux-sens) ou ledit codon stop, ou à la partie complémentaire de celle-ci.

**17.** Anticorps ou aptamère ou phage liant de façon spécifique un produit protéique du gène *RHD* selon la revendication 15.

**18.** Méthode pour tester la présence d'une molécule d'acide nucléique codant un antigène Rhésus D mutant tel que **caractérisée par** la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 14 dans un échantillon comprenant l'hybridation de l'oligonucléotide selon la revendication 16 dans des conditions stringentes à des molécules d'acide nucléique comprises dans l'échantillon obtenu à partir d'un humain et la détection de ladite hybridation.

**19.** Méthode selon la revendication 18, comprenant en outre la digestion du produit de ladite hybridation avec une endonucléase de restriction et l'analyse du produit de ladite digestion.

**20.** Méthode pour tester la présence d'une molécule d'acide nucléique codant un antigène Rhésus D mutant tel que **caractérisée par** la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 14 dans un échantillon, comprenant la détermination de la séquence d'acide nucléique d'au moins une partie de la structure moléculaire d'acide nucléique ou de la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 14, ladite partie codant ladite mutation (faux-sens) ou ledit codon stop.

**21.** Méthode selon la revendication 20 comprenant en outre, avant la détermination de ladite séquence d'acide nucléique, l'amplification d'au moins ladite partie de ladite molécule ou structure d'acide nucléique.

**22.** Méthode pour tester la présence d'une molécule d'acide nucléique codant un antigène Rhésus D mutant tel que **caractérisée par** la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 14 dans un échantillon, comprenant la réalisation d'une réaction d'amplification en utilisant un ensemble d'amorces qui amplifie au moins une partie de ladite séquence, dans laquelle au moins l'une des amorces utilisées dans ladite réaction d'amplification est l'oligonucléotide selon la revendication 16.

**23.** Méthode selon la revendication 21 à 22, dans laquelle ladite amplification est réalisée par, ou ladite réaction d'amplification est, l'amplification en chaîne par polymérase (PCR).

**24.** Méthode selon la revendication 23, dans laquelle ladite PCR est une PCR-RFLP, PCR-SSP ou PCR longue distance.

**25.** Méthode selon l'une quelconque des revendications 21 à 24, dans laquelle le poids moléculaire du produit d'amplification est analysé.

**26.** Méthode pour tester la présence de la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 14 codant des allèles positifs vis-à-vis de *RHD*, comprenant les étapes suivantes :

(a) isolement de l'ADN provenant d'un échantillon de sang ou d'un donneur de sang ;
(b) hybridation d'au moins deux amorces orientées de manière opposée, dans des conditions stringentes, à l'ADN de manière à réaliser une PCR ;
(c) amplification de la séquence cible ;
(d) séparation des produits d'amplification sur un gel ; et
(e) analyse des amplicons,

dans laquelle lesdits allèles positifs vis-à-vis de *RHD* sont dérivés à partir d'un échantillon RhD négatif sur le plan

sérologique.

**27.** Méthode selon la revendication 26, dans laquelle ledit échantillon est choisi à partir d'une population Caucasienne.

**28.** Méthode pour tester la présence d'un produit protéique du gène *RHD* selon la revendication 15 dans un échantillon comprenant l'évaluation d'un échantillon obtenu à partir d'un humain pour la liaison spécifique avec l'anticorps ou l'aptamère ou le phage selon la revendication 17.

**29.** Méthode pour tester la présence d'un produit protéique du gène *RHD* codant la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 5 dans un échantillon comprenant l'utilisation des méthodes d'agglutination directe, des tests indirects à l'antiglobuline, des anticorps monoclonaux anti-D et/ou des techniques d'adsorption/ élution.

**30.** Méthode selon l'une quelconque des revendications 18 à 29, dans laquelle ledit échantillon est du sang, sérum, plasma, tissu foetal, salive, urine, tissu muqueux, mucus, tissu vaginal, peau, cheveu, follicule capillaire ou autre tissu humain.

**31.** Méthode selon la revendication 30, comprenant l'enrichissement en cellules foetales ou l'extraction de l'ADN ou l'ARNm foetal, à partir du tissu maternel, comme le sang périphérique, le sérum ou le plasma.

**32.** Méthode selon l'une quelconque des revendications 18 à 31, dans laquelle ladite molécule d'acide nucléique ou ledit matériel protéique provenant dudit échantillon est fixé à un support solide.

**33.** Méthode selon la revendication 32, dans laquelle ledit support solide est une puce.

**34.** Utilisation de la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 14 pour l'analyse d'un phénotype Rhésus D négatif ou positif.

**35.** Utilisation de la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 14 ou du produit protéique du gène *RHD* selon la revendication 15 pour l'évaluation de l'affinité, l'avidité et/ou la réactivité d'anticorps monoclonaux ou d'antisérums polyclonaux, de préférence des antisérums-D, des antisérums anti-globuline ou anti-globuline-humaine.

**36.** Utilisation de cellules, de préférence des globules rouges de proposants portant la molécule d'acide nucléique selon l'une quelconque des revendications 1 à 14 pour l'évaluation de l'affinité, l'avidité et/ou la réactivité d'anticorps monoclonaux anti-D ou d'antisérums polyclonaux anti-D, anti-globuline ou anti-globuline-humaine, ou de préparations de ceux-ci.

**37.** Méthode de caractérisation des anticorps monoclonaux ou des antisérums polyclonaux ou des préparations de ceux-ci, ladite méthode comprenant

(a) le test d'une molécule d'acide nucléique d'un échantillon d'un proposant pour la présence d'une mutation telle que définie dans l'une quelconque des revendications 1 à 14 ;
(b) la corrélation, sur la base de la mutation et du statut allélique du gène *RHD,* de l'acide nucléique avec la densité du produit protéique du gène *RHD* sur la surface des globules rouges dudit proposant ;
(c) la réaction desdits anticorps monoclonaux ou antisérums polyclonaux ou de ladite préparation de ceux-ci avec une cellule portant le produit protéique du gène *RHD* à sa surface ;
(d) la caractérisation desdits anticorps monoclonaux ou antisérums polyclonaux ou de ladite préparation de ceux-ci sur la base des résultats obtenus à l'étape (c).

**38.** Méthode selon la revendication 37, dans laquelle ladite caractérisation comprend la détermination de la réactivité, la sensibilité, l'avidité, la spécificité d'affinité et/ou d'autres caractéristiques des anticorps et antisérums.

**39.** Méthode selon la revendication 37 ou 38, dans laquelle ladite cellule portant le produit protéique du gène *RHD* à sa surface est un globule rouge.

**40.** Méthode pour déterminer si un patient nécessitant une transfusion sanguine doit être transfusé avec du sang négatif vis-à-vis de RhD provenant d'un donneur, comprenant l'étape de tester un échantillon provenant dudit patient vis-

à-vis de la présence d'une ou plusieurs molécules d'acide nucléique selon l'une quelconque des revendications 1 à 14, dans laquelle un test positif pour deux desdites molécules d'acide nucléique différentes indique le besoin d'une transfusion avec du sang négatif vis-à-vis de Rh.

5

**41.** Méthode pour évaluer le risque pour une mère RhD négative de concevoir ou porter un foetus RhD positif, ou du risque pour une mère ayant un titre anti-D de concevoir ou porter un foetus risquant de développer une maladie hémolytique du nouveau-né, comprenant l'évaluation d'un échantillon obtenu à partir du père du foetus pour la présence d'une ou plusieurs molécules d'acide nucléique telles que définies dans l'une quelconque des revendications 1 à 14.

10

**42.** Préparation comprenant l'anticorps ou l'aptamère ou le phage selon la revendication 17.

**43.** Méthode d'identification d'une chaîne $V_H$ ou $V_L$ d'anticorps ou une combinaison de celles-ci, ou un aptamère liant de façon spécifique un produit protéique du gène *RHD* selon la revendication 15 comprenant

15

(a) la mise en contact du produit protéique du gène *RHD* selon la revendication 15 avec une banque de phages présentant des chaînes $V_H$ ou $V_L$ ou des combinaisons de celles-ci à la surface du phage ou avec des aptamères ;
(b) l'identification du phage ou d'aptamères qui lient ledit produit protéique; et éventuellement
(c) la répétition des étapes (a) et (b) une ou plusieurs fois.

20

**44.** Méthode d'identification d'un anticorps monoclonal liant de façon spécifique un produit de protéine du gène *RHD* selon la revendication 15 comprenant

(a) la mise en contact du produit protéique du gène *RHD* selon la revendication 15 avec un ou plusieurs anticorps monoclonaux ;
25
(b) l'identification des anticorps monoclonaux qui lient ladite protéine ; et éventuellement
(c) la répétition des étapes (a) et (b) une ou plusieurs fois.

**45.** Méthode d'identification d'une chaîne $V_H$ ou $V_L$ d'anticorps ou une combinaison de celles-ci, ou un aptamère liant de façon spécifique un produit protéique du gène *RHD* selon la revendication 15 comprenant
30

(aa) la mise en contact du produit protéique; et
(ab) d'un polypeptide D normal
dans laquelle le polypeptide D normal est présent en une masse molaire qui est supérieure, égale ou inférieure au produit protéique du gène RHD selon (aa)
35
avec une banque de phages présentant des chaînes $V_H$ ou $V_L$ ou des combinaisons de celles-ci à la surface du phage ou avec des aptamères ;
(b) l'identification du phage ou d'aptamères qui lient ledit produit protéique du gène *RHD* selon (aa) ; et éventuellement
40
(c) la répétition des étapes (a) et (b) une ou plusieurs fois.

**46.** Méthode d'identification d'un anticorps monoclonal liant de façon spécifique un produit protéique du gène *RHD* selon la revendication 15 comprenant

45
(aa) la mise en contact du produit protéique du gène *RHD* ; et
(ab) d'un polypeptide D normal
dans laquelle le polypeptide D normal est présent en une masse molaire qui est supérieure, égale ou inférieure au produit protéique du gène *RHD* selon (aa)
avec un ou plusieurs anticorps monoclonaux ;
50
(b) l'identification des anticorps monoclonaux qui lient ledit produit protéique du gène *RHD* selon (aa) ; et éventuellement
(c) la répétition des étapes (a) et (b) une ou plusieurs fois.

**47.** Méthode selon l'une quelconque des revendications 43 à 46, dans laquelle le produit protéique du gène *RHD* est exposé à la surface d'une cellule.
55

**48.** Méthode selon l'une quelconque des revendications 43 à 47, dans laquelle le polypeptide ou la cellule hôte est fixé à un support solide.

**49.** Méthode selon l'une quelconque des revendications 43 à 48, dans laquelle, à la suite de l'étape (b) ou (c), l'étape suivante est réalisée :

(d) l'identification de la séquence d'acides aminés des chaînes $V_H$ ou $V_L$ et/ou l'identification de la séquence d'acide nucléique codant ladite séquence d'acides aminés.

**50.** Méthode selon l'une quelconque des revendications 43 à 46, dans laquelle, dans le cas où un seul cycle de sélection est utilisé pour l'identification, le nombre de molécules protéiques du gène *RHD* selon (a) est en excès molaire par rapport au nombre de particules de phage.

**51.** Utilisation de cellules, de préférence des globules rouges comprenant le produit protéique du gène *RHD* selon la revendication 15, à partir de proposants pour l'évaluation de l'affinité, l'avidité et/ou la réactivité d'anti-D monoclonaux selon la revendication 17 ou d'anti-D polyclonaux ou d'antisérums anti-globuline ou anti-globuline-humaine ou de préparations de ceux-ci.

**52.** Kit comprenant

(a) l'oligonucléotide selon la revendication 16, et/ou
(b) l'anticorps selon la revendication 17 ; et/ou
(c) l'aptamère selon la revendication 17 ; et/ou
(d) le phage selon la revendication 17 ; et/ou
(e) une paire d'amorces utile pour réaliser la réaction d'amplification selon l'une quelconque des revendications 20 et 25.

Fig. 1

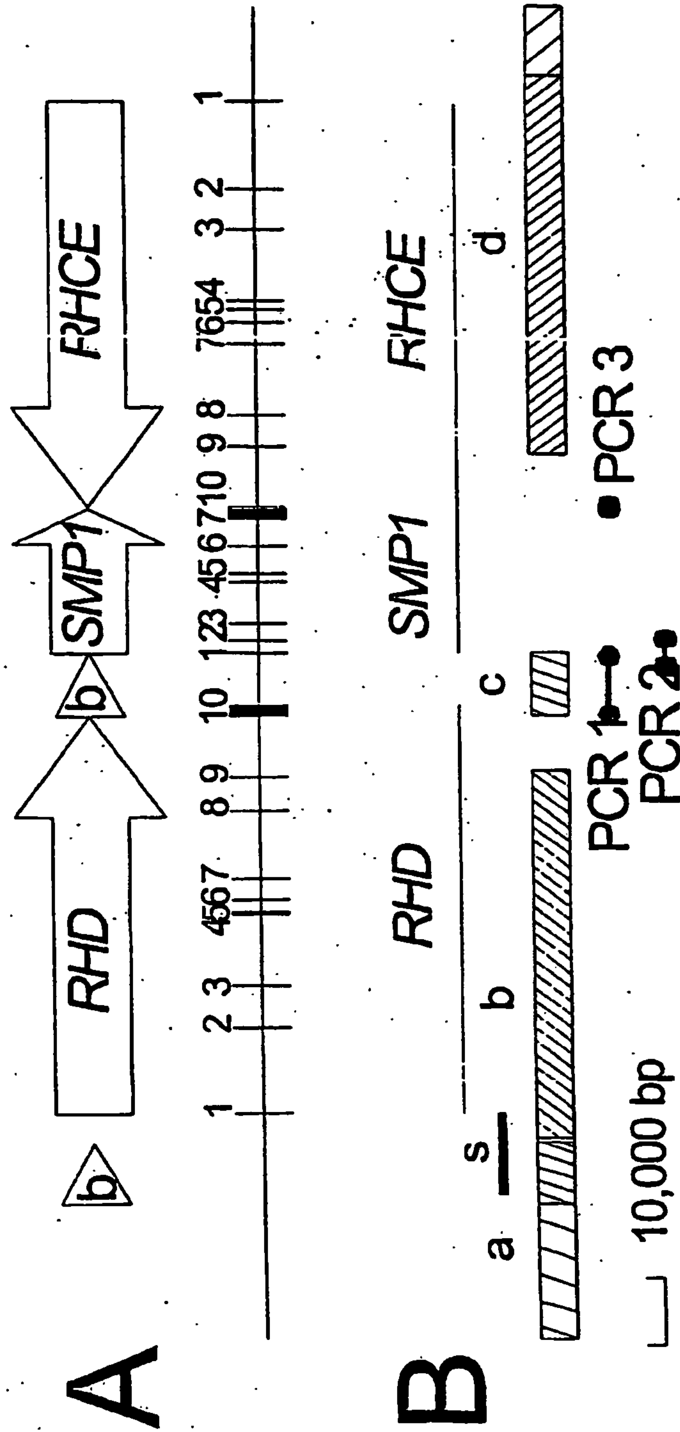


Fig. 2

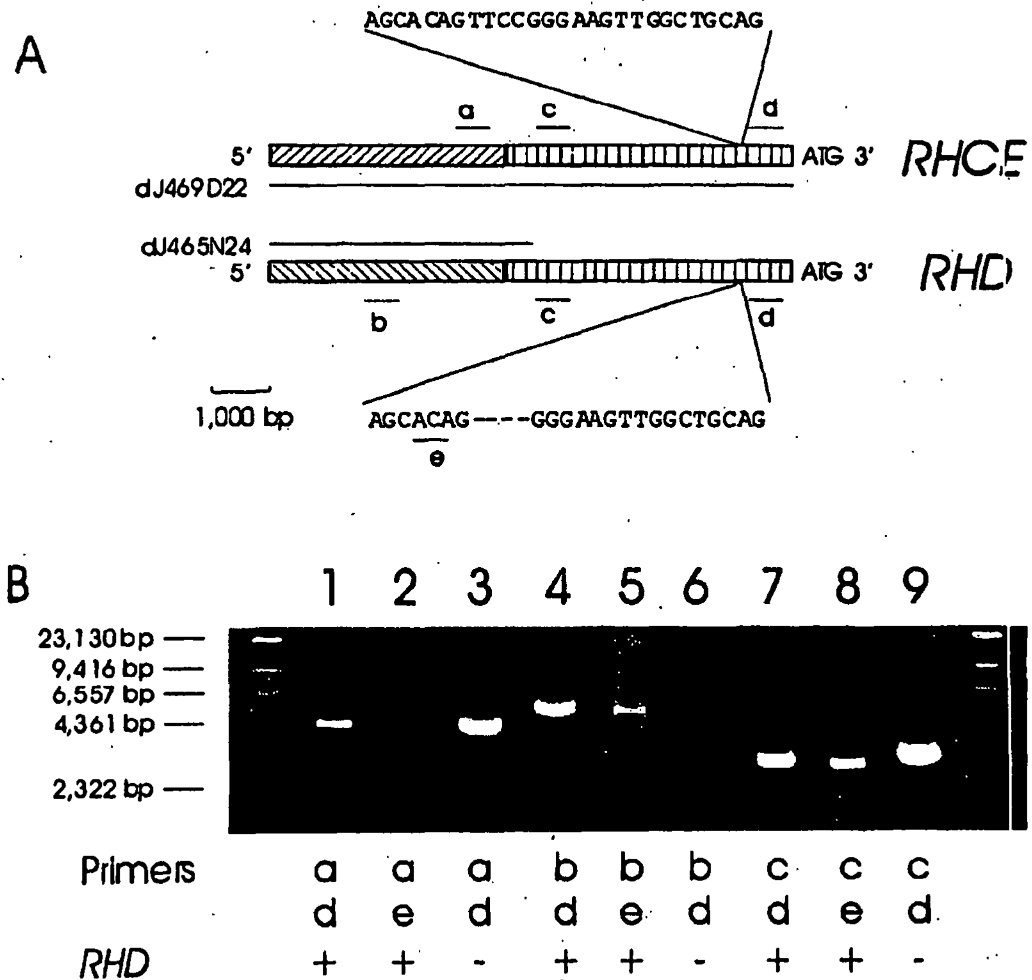


**Fig. 3**

```
-- Rhesus box ------------------------------------->
gcatgcgcgactgagccgggtggatggtactgctgcatccgggtgtctg

              <- SMP1 Exon 1 (91 bp, 5'UTR)-------
gaggctgtggccgttttgttttcttggctaaaatcgggggagtgaggcggg

-- SMP1 Exon 1 ----------->&lt;--- Intron ~ 2,000 bp
...actgcacgacggggctggactgacgt.....................

Intron --->&lt;---- SMP1 Exon 2 (106 bp, 93 translated)-----
........agctgaaaaaaATGTCTGGATTTCTAGAGGGCTTGAGATGCTCAG...

-- SMP1 Exon 2 ----------->&lt;--- Intron ~ 2,500 bp
...GCTTCCATTGCTGCTGGTGTACTAgt...

Intron ---->&lt;--- SMP1 Exon 3 (113 bp, all translated)----
..........agTTTTTACAGGCTGGTGGATTATCATAGATGCAGCTGTTATT...

-- SMP1 Exon 3 ----------->&lt;--- Intron ~ 8,000 bp
...TATAGCAACCATAGCCTTCCTAATgt.....................

Intron ---->&lt;--- SMP1 Exon 4 (68 bp, all translated)-----
..........agGATTAATGCAGTATCGAATGGACAAGTCCGAGGTGATAGTTA...

-- SMP1 Exon 4 ----------->&lt;--- Intron ~ 1,400 bp
...TGAAGGTTGTCTGGGTCAAAACAGgt.....................
```

**Fig. 3 cont.**

```
Intron ---->&lt;--- SMP1 Exon 5 (93 bp, all translated)-----
..........agGTGCTCGCATTTGGCTTTTCGTTGGTTTCATGTTGGCCTTTG...

-- SMP1 Exon 5 ----------->&lt;--- Intron ~ 4,000 bp
...TTTTTGGAGGTTATGTTGCTAAAGgt.....................

Intron ---->&lt;--- SMP1 Exon 6 (61 bp, all translated)-----
..........agAAAAAGACATAGTATACCCTGGAATTGCTGTATTTT.........

-- SMP1 Exon 6 ----------->&lt;--- Intron ~ 4,000 bp
...TCCAGAATGCCTTCATCTTTTTTGgt

Intron ---->&lt;--- SMP1 Exon 7 (1,703 bp, 47 translated)---
..........agGAGGGCTGGTTTTTAAGTTTGGCCGCACTGAAGACTTATGGCAGT

                  &lt;----------- RHCE Exon 10 -----
-- SMP1 Exon 7 ----------------------------------
GAacac....agcatcatcctaatgaaactaaacatttattttaaac

----------- RHCE Exon 10 ----------------------------
-------- SMP1 Exon 7 ---&gt;
ttattaaattgactcttaaactaagttttagtctttaatttttaatatcaa

                              &gt;---- Homology with RHD -
-- RHCE Exon 10 ---------------------------------------
atctgtctctgaccttgtttcattatacataaggagctttgctgtcatgagcgtttc
```

**Fig. 3 cont.**

```
---------- Homology with RHD --------------
------------- RHCE Exon 10 ---------------
tcacgtacaaatgcaggcaacagtgagaggaagttgtcttgtt

-- Homology with RHD --------------------------------------
-- RHCE Exon 10 -------------------------------------------
tttgaacaggccttgtttttcttggatgcttttgcTTAAAATCCAACAGCCAAATGAGG
```

Fig. 4

**Fig. 5**

```
attcactatcacaagaacagcacgggtaa
*****************************
**-*********g****************

  -------rez7----------->
gacctgtccccatgattcagttacct
**************************
**************************

cccactgggtccctcccacaacgcatgggaattcaggatgagattt
**********************************************
**********************************************

gggtggggacacaaccaaaccctatcattccacccatggcccctcccaaa
**************************************************
**************************************************

tttcatgtcctcacatttcaaaaccaatcacaccatcccaacagtccctc
**************************************************
**************************************************

aaagtcttaaatgatttcagcattaactcaaaagtccacagtctaatgtc
**************************************************
**************************************************
```

**Fig. 5 cont.**

```
tcatctgagacaaggcaagtcctttccatttatgagcctataaaatccaa
**************************************************
*****************************c********************

agcaagttagttacttcctagatacaatgggggtacaggcattgggtaaa
**************************************************
*********a****************************************

tacagccattccaaatgggataaattggtcaaaacaaagaggctacaggc
**************************************************
**************************************************

ccatgagagtccaaaatccagtggggcagtcaaatcttaaagctccaaaat
***************************************************
***************************************************

gatctcct-ttgactccacatctcacatccaggtcacgcagatggaagg
********-****************************************
********c****************************************

ggtgggttcccatggtcttgggcagctctgcccctgtacctttgcagggt
**************************************************
**************************************************
```

**Fig. 5 cont.**

```
acagcctccctctcagctgctttcatgggctggcattgagtgtctgcagc
**************************************************
************************************************a*
                                           ^^^^^^

ttttccaggtacacggtgcaagctgtcggtggatctaccattctggggtc
**************************************************
**************************************************

tggaggacctcttctcacagctccactaggtggtgccccagtagggactg
**************************************************
**************************************************

tgtgtggggtctctgaccccacatttcccttctgcactgccctggcagag
**************************************************
**************************************************

gatctccatgagggccctgctcctgcagcaaacttctgactgggcatcca
**************************************************
********************c*****************c***********
                      ^^^^^^

ggcatttccgcacatcctctttaatctaggcgaaggtttccaaaccccaa
**************************************************
*************************************************g
```

**Fig. 5 cont.**

```
ttcttgacttctgtgcactcgcagtctcaacaccacatggaagctgtcaa
**************************************************
**************************************************

ggcttggggcttgcactccccgaagctacagcccaagctctaccttgcct
**************************************************
**************************************************

cccgtcagtcatggttgggagtggctgggatgcagggcaccaagtcccta
**************************************************
**t***********************************************

ggctgcacacagcatgaggaccccggcctggccaacaaaccatttttt
**************************************************
**************************************************

        +-- breakpoint region ->
        +-- identity region ->
cctgatacctctggacctgtgatgggaggggttgccataaagacctctga
**************************************************
*******t******************************************

catgccctggagacattttccccattgtcttgggaattagcatttggctc
**************************************************
**************************************************
```

**Fig. 5 cont.**

```
ctgttactcatgcaaatttctgcagccagcttgaatttctcctcagaaaa
**************************************************
**************************************************
                   ^^^^^^

tgggaattttcttttctatcacattgtcaggctgcaaattttccgaactt
**************************************************
**************************************************

ttatgctctgcttcccttataaaactgaatgtctttaacagcacccaag
*************************************************
*************************************************

tcacctcttgaatgctttgctgcttagaaatttctcctgccagatactct
**************************************************
**************************************************

aaatcatctctctgaagttcaaagttctacaaatatctcgtgcaggggca
**************************************************
**************************************************

aaatgccgccagtatctttgctaaaacataacaagagtcccctttgctcc
**************************************************
**************************************************
```

**Fig. 5 cont.**

```
agttcccaacaagttcctcatttccgtctgagaccacctcagcctatgga
**************************************************
**************************************************

ctttattgtccacagtgctatcagcatttgggcaaagccattcaacaag
**************************************************
**************************************************
tctctaggaagttccaaactttcccacatttgcctgtcttcttctgagcc
**************************************************
**************************************************

ctccaaactgttccaaaccctgcctgttacccagttccaaagtcacatac
**************************************************
**************************************************

ccattttgagtatctacggcagcaccccactctactggtaccaatttag
**************************************************
**************************************************

ccactgaagtagttggagaacagaagtaatagactctggtttacattgta
**************************************************
**************************************************

aaagcttctctgtggctgctgtgtgaagaaatatatgagaatgaagccc
**************************************************
**************************************************
```

**Fig. 5 cont.**

```
caagatgaagcagggacacagttgcagtggttagagtaagaaatgctgct
**************************************************
**************************************************

ggctggcactgaagtgatagcctggaggtttgtgtgcacatgcatgtg
************************************************
************************************************

tatgtgttttacgatagtaggcccaacagat
*******************************
*******************************

  <-- breakpoint region ----+
actgtaatccacacttgtttttttttttt----ga
*****************************tttt**
*****************************tttt**

gacagagtctcacctgttgcctagactagaatgc
**********************************
**********************************

agtggcacaatcttggctcactacaacctccacctcccaggttcaaacaatc
****************************************************
****************************************************
```

**Fig. 5 cont.**

cttgtgcttcagcctcccgagtagttgggattacaggtgtgtgccacc
************************************************
************************************************

gtgcccagctatatttttgtatttttagcagagatgggatttgccacat
*************************************************
*************************************************

tggccaggctggtcttgaactcctggcctcaagcaatcctcccacctta
*************************************************
*************************************************

gcctcccaaagtgctgagccaccacacctggccgcaactgattttaatc
*************************************************
*************************************************

atgaaatgacacatacatttaaaaacccaatacctataatattcctggc
*************************************************
*************************************************

tagtactcttcacatctatatcatcaaaacaaagaaagtatgtgaaact
*************************************************
*************************************************

**Fig. 5 cont.**

```
gacacagccaaggggagactaaggagacataacaattaactgtaatgtgg
**************************************************
**************************************************

tattctggaggggatcctggaacagaaaaagacattaggcaaaaaactaa
**************************************************
**************************************************

agaaatctgaataaaatgtggatgtcagttaataataatgtatcatatta
**************************************************
**************************************************

    <-- identity region-+
gtccagtaattgtaacaaatataccacaataatgaaagccattaattata
*************************-************************
*************************-************************

gggaaaatggaggggttaatatgggtggctggctttgctatttctagcag
**************************************************
**************************************************

ctccatttatctgcaaaagacaaacattcattaagtcccaaaaaggtaaa
*************a************************************
*************a************************************
```

**Fig. 5 cont.**

```
gaatgacaaattaagcatgtatcttattagtaagagtaatataaagatg
*************************************************
*************************************************

ctcactcctatttataaatatttgacaatcatgttaaggccacaaaagag
*******a********************g************g********
*******a********************g************g********

aaaaaagggtaggggcaaaaaacgcaaagagaaaggagttagtatctttt
**************************************************
**************************************************

ctcccgcactcattagctattaaaagaggatgtttgtttaaagctgctca
**************************************************
**************************************************

gagctggtaaactaatgttaagtcactaacgggaatttaaaaggtttcat
**************************************************
**************************************************

taagaactgcctgcactagattcctccaccctgagacattaaacaatcac
**************************************************
**************************************************
```

**Fig. 5 cont.**

```
gataaacctcctgagtggtaagaacttgtccatttaaaaacaggctatag
*************************g************************
*************************g************************

attgtatcatgcagttttatctactaatcggctaa
*****--****************************
*****--****************************

tatcccgccaaaaac----aaaaaccccaaa
*gca**********aaac******a******
*gca**********aaac******a******
 <--------rnb31-----------mm

gggatgaaagtttcatccatcaaaggaaacaac
*********************************
*********************************
```

Fig. 6

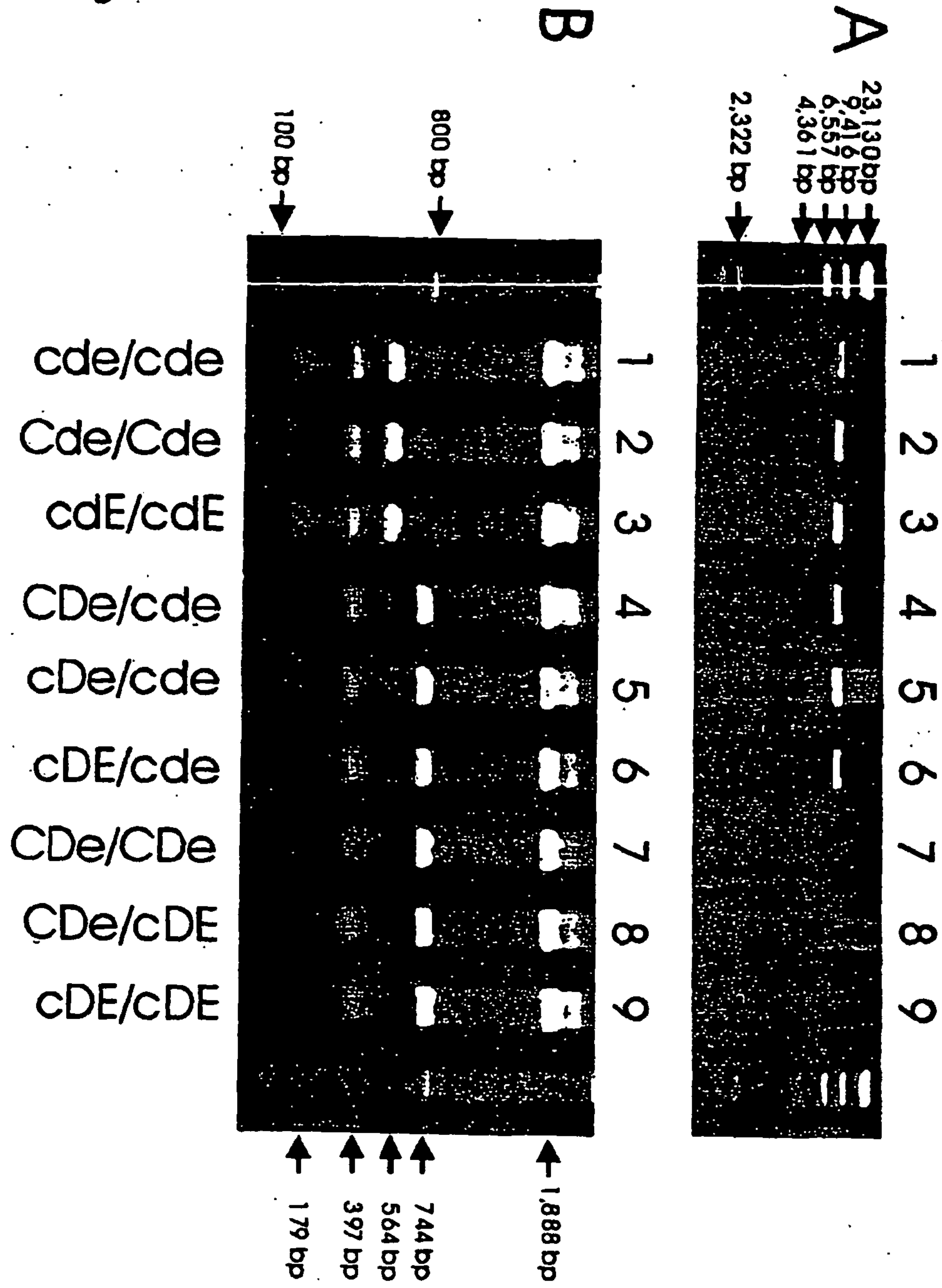
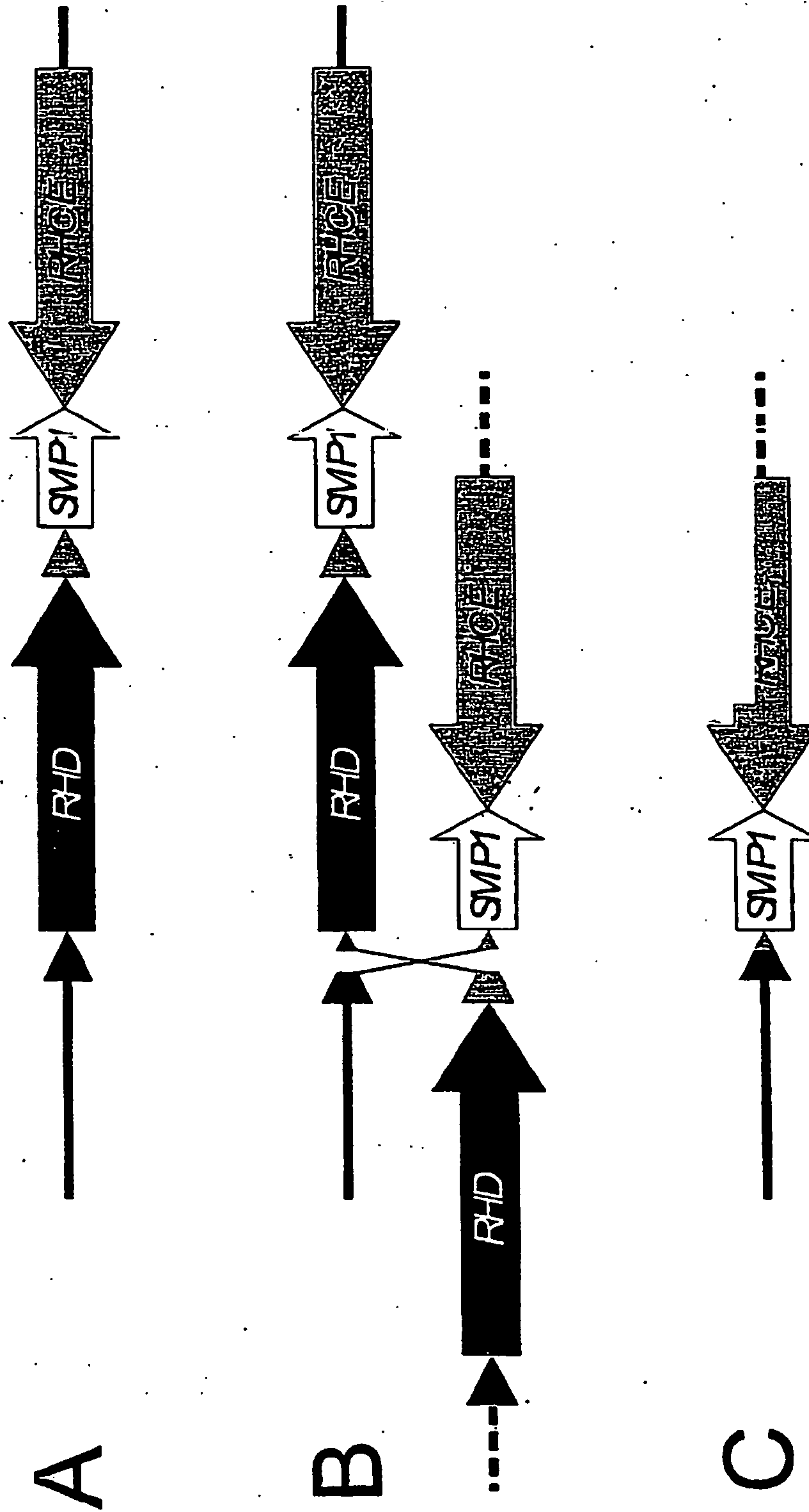


Fig. 7



# Fig. 8

Hybrid Rhesus box of RHD negatives

```
5' ctagaaaacactttgtcattttagaggtgtta
(start of Rhesus box)
tccaatgttcgcgcaggcactggagtcagagaaaatggagttgaatcctttctctgccactc
tttgaggagaatctcaccatttattatgcactgtagaatacaacaataaaatacagccatgt
accacataacaacatcttggtaaacaacagactgcatatatgatggtggtcatccagtaagc
taaggttaatttattattattccctttttttttcttttttttgagatgtagtcttactctg
tcacccaggctagagtgcaatggcaccatcttggctcactgcaacctctgcctcctgggttc
aagcgaatctcctgcctcagcctcccgaagtagctgggattacaggcacccaccacatctgg
ctaatttttgtatttttagtaaagatggggtttcaccatgttggccaggctgatctcaaac
tcctgacctcaagtgatctgcctgcctcggcctcccaaagtgctgggaccataggcctgagc
cactgtgcccggccttgtttgctttttaacagttaacagtgtgctcatagaaactgctttg
acatgactgcaatcatgtgcttcatagaaacttaattagattataccactagagtcttcaga
ttttatactttttttttgaaacggagtctcactctgtcaccaggctggagtgcagtgccg
caatctcagctcgccgcaacctccgcctcccaggttcaagtgattctcctgcctcagcctcc
cgagtagctgggattacaagtgcacactaccacgcccagctaattttgcatttttactaga
cagggtttcaccatgttggctaggatagtttcaccaggatctcttggcctcatgatcagcct
gcctcggcctcccaaagtgctgggattacaggtgtgagccaccgtgcccagcctatacttcc
cttttgaataccatttggcgttttgaagaattaacagctttgtgaacgtggcagtgcttgt
gattcaggcttccactgagaccaaggggagaacctggttgcaggacaaacagacggacagcg
tgtggcagtgtttaaatgctcttctgaaggctgatacgacagctctctgtgcactgattgta
tacgcatcccaagattatattattgttttctattgctatgtgtcacactttgccaaacagga
tgtggaaaatgaataagcggttttcttaggcacttcttaacagacaattggtcaaaatgaac
tccattgcttaagaaacacataaacaccatttagtcactgaatatagctatatgtatggttg
ctactatggggaatcttgttttgccaatttctttgaaaattctggcagaccaaggttcttt
ttgtttacacaatacttgaaaaataaaaatgaacaagccaacaaactaccaagttttcactt
acataaatgtagttacatacagaaaatgtgactgtgaatttttctaggactttaaactat
aagcactatttgcacgaaagagaaccaatctatcaattacaaactcacataattttacagat
ttttttttccctacacagcacataaaacagaaggaatttgaagccaccctccaaacacaggg
gaaggaggctgtgtgtatatcctcattgtctttcacattctaaggtggttccactcagtgac
tgaaatccttaagtgttgtattagtcggcttgggctaccataacagcagcttaaactgttgt
taagccactcagacttaaacaacagaaatttatttccttatagttctggaggctggaagttc
aaggtgccggcaaggctggtttctggtgagacctctctccctgtcttgcagatggctgcctc
ctccctgtgtcctcatagagcctgtcctctgcttttacacttctggtgtcatcttcctttttt
tttttttttgagacagagtctcgctctatcgcccaggctggagtgcagtggcccgatcga
tctcggctcactgcaacctctgcctcccaggttcaagcaattctcctgcctcagcctcccga
gtagctgggactacaggtgcccgccatcatgtctggctaattttgtatttttagtagagac
agggtttcaccatattggccaggctggtctccaactcctgaccttgtcatctgcctgcctcg
gcctcccaaagtgctaggattacaggcgtgagccaccgcacccggcctctttctcttcttat
aaggacaccagtcctattagattagggctccaccctcatgacctcatttgaccttaactatt
atttctttaaagcacctatttccaaatatagtcactttaggggttagggcttcaaaatatga
atctgagggagatcaattcagtaaatagcagtagtcattaacggacaatatatacaaagata
atttcgtgattactgtccttatgcataaatgtcctcagtgttccactgcctttatccagatt
tactatcacaaagactttgctctgagaaaaatgtgatttctttctttttttttttttttga
gacagagtctcactctgtcacccaggctggagtgcagtggtgcaatctcggctcactgcaat
ctccgcctcccaggttcacgccattctcttgcctcagtctcccgagtagctgggcctacagg
```

**Fig. 8 cont.**

Hybrid Rhesus box of RHD negatives

cgcccgccaccctgcccagctaatttttgtatttttagtagagacggggtttcaccatgtt
agccaggatggtctcaatctcctgacctcgtgatccacctgcctcagcctcccaaagtgctg
ggattacaggcatgagccaccgcgcccagcagattttttttttttttttttttgagat
ggagtcttgctgtgttgcccagcctggagtgcagtgttatgattttggctcactgcaacctc
tgtctaccatgttcaagcgattctcccacctctgcctcccgtgtagctgggatcacaggcac
acgccaccacacctagctactttttgtatttttagtagaaatggggtttcaccatgttggcc
aggatggtcccgaactcctgacctcaagtgatcctcctgcctcggcctcccaaagtgctggg
attacaggtgtgagccactgtgcctggccaaaaatgtgatttctttatttcccacattgccaa
ttccatttcaattaactataatagctatgtctattgagcactcaagtgtattctagaaactg
ttcctgattctggggatatatccatgaatcaactatagtccctgttattaagtaatctgtag
tctgactaaaccattagaaatttaaaaaatggctactttcaaagacatcttggagttcagga
gtcccacactgcgaaccatattacctaataatccaacctgcttgtaattcacttatttaacc
aatatttattgagtgccaactttgagcctaagatacagcagtaaacaaatggataaagtccc
tgtcctcatgaaacttgtattctaatggaagaaacagaaaacaaacagatataggatgtaat
atcaggtagggataaatactttgaattcaaacaaaagtatacgtagtcagggttcgccaaag
agacacagccaatcggatacatagatatataaaagagggtttatgagttagaaagggctcac
atgattacagaggctgagaagtcccacagcagattgtctgcaagctggagacccagggatac
tggtagcatggctcagtccaagtcccaaagcctcagaatcaggaaagctgatgatataattc
ttagcccaaaggccttagaaccccagcggtgacggaaggctgatgtaggtcctggagtcct
gagacccaacagcctgggatcctgaaatccaagggcaggaatggaagcgtgtattccagctc
caagagagtaagaccaatttgcctttcttccgttttgtttcaagccacctgcacattgagg
gcggatggttccctcttagtccattcagtcatatatcaatctcttctggaaataccctcaca
gacacactaacaaataatgcctttccagttctctaggtattctttaatccagtcaagctgac
acctaaaattaaccatcacaaaagttaaggagaaagaagacaacttgtaggggaggctgcta
tgcaagacagtgtgtgaaggaagggctctctgaagaggttaatatctgagcagagacttgaa
tgaagtgaagaagtgagccatgtgggtatggggaatacaacttccaggtagagaagacaagt
gtggtgtgtatcagggtcagcaaagaagccatgtgacagagaagggtgggccagggagagac
ggataagtgatctaactcctgaggaggtggcctggccaggagctagagcatgaagatctcgt
aggactttattctgcaaggtgaaaagccattgtattagtctgttcacaaacccgagactagg
caatttacaaaagaaagagaggtttaatggacttacagttccacatggctggggaggcctca
caatcatggcgaaaggcaatgaggagcaagtcacgtcttacgtggatggcaggcaaagacaa
agacagcttgtgcagagaaactccccсttatagagccatcagatcctgttagacttattcac
tatcacaagaacagcacgggtaagacctgtccccatgattcagttacctcccactgggtccc
tcccacaacgcatgggaattcaggatgagatttgggtggggacacaaccaaaccctatcatt
ccacccatggcccctcccaaatttcatgtcctcacatttcaaaaccaatcacaccatcccaa
cagtccctcaaagtcttaaatgatttcagcattaactcaaaagtccacagtctaatgtctca
tctgagacaaggcaagtcctttccatttatgagcctataaaatccaaagcaagttagttact
tcctagatacaatggggggtacaggcattgggtaaatacagccattccaaatgggataaattg
gtcaaaacaaagaggctacaggcccatgagagtccaaaatccagtggggcagtcaaatctta
aagctccaaaatgatctcctttgactccacatctcacatccaggtcacgcagatggaagggg
tgggttcccatggtcttgggcagctctgccccctgtacctttgcagggtacagcctccctctc
agctgctttcatgggctggcattgagtgtctgcagcttttccaggtacacggtgcaagctgt
cggtggatctaccattctggggtctggaggacctcttctcacagctccactaggtggtgccc
cagtagggactgtgtgtggggtctctgaccccacatttcccttctgcactgccctggcagag
gatctccatgagggccctgctcctgcagcaaacttctgactgggcatccaggcatttccgca

**Fig. 8 cont.**

**Hybrid Rhesus box of RHD negatives**

catcctctttaatctaggcgaaggtttccaaaccccaattcttgacttctgtgcactcgcag
tctcaacaccacatggaagctgtcaaggcttggggcttgcactccccgaagctacagcccaa
gctctaccttgcctcccgtcagtcatggttgggagtggctgggatgcagggcaccaagtccc
taggctgcacacagcatgaggaccccgggcctggccaacaaaccatttttcctgatacct
ctggacctgtgatgggaggggttgccataaagacctctgacatgccctggagacattttccc
cattgtcttgggaattagcatttggctcctgttactcatgcaaatttctgcagccagcttga
atttctcctcagaaaatgggaattttcttttctatcacattgtcaggctgcaaattttccg
aacttttatgctctgcttcccttataaactgaatgtctttaacagcacccaagtcacctct
tgaatgctttgctgcttagaatttctcctgccagatactctaaatcatctctctgaagttc
aaagttctacaaatatctcgtgcaggggcaaaatgccgccagtatctttgctaaaacataac
aagagtccccttttgctccagttcccaacaagttcctcatttccgtctgagaccacctcagcc
tatggactttattgtccacagtgctatcagcattttgggcaaagccattcaacaagtctcta
ggaagttccaaactttcccacatttgcctgtcttcttctgagccctccaaactgttccaaac
cctgcctgttacccagttccaaagtcacatacccattttttgagtatctacggcagcacccca
ctctactggtaccaatttagccactgaagtagttggagaacagaagtaatagactctggttt
acattgtaaaagcttctctgtggctgctgtgtgaagaaaatatatgagaatgaagccccaag
atgaagcagggacacagttgcagtggttagagtaagaaatgctgctggctggcactgaagtg
atagcctggaggtttgtgtgtgcacatgcatgtgtatgtgttttacgatagtaggcccaaca
gatactgtaatccacacttgtttttttttttttttgagacagagtctcacctgttgcctag
actagaatgcagtggcacaatcttggctcactacaacctccacctcccaggttcaaacaatc
cttgtgcttcagcctcccgagtagttgggattacaggtgtgtgccaccgtgcccagctatat
tttttgtatttttagcagagatgggatttgccacattggccaggctggtcttgaactcctg
gcctcaagcaatcctcccaccttagcctcccaaagtgctgagccaccacacctggccgcaac
tgatttttaatcatgaaatgacacatacatttaaaaaacccaatacctataatattcctggc
tagtactcttcacatctatatcatcaaaaacaaagaaagtatgtgaaactgacacagccaag
gggagactaaggagacataacaattaactgtaatgtggtattctggaggggatcctggaaca
gaaaaagacattaggcaaaaaactaaagaaatctgaataaaatgtggatgtcagttaataat
aatgtatcatattagtccagtaattgtaacaaatatacccaataatgaaagccattaattat
agggaaaatggaggggttaatatgggtggctggcttttgctatttctagcagctccatttta
tctacaaaagacaaacattcattaagtcccaaaaaggtaaagaatgacaaattaagcatgta
tcttattagtaagagtaatataaagatgctcactcatatttataaatatttgacaatgatgt
taaggccagaaaagagaaaaaagggtaggggcaaaaaacgcaaagagaaaggagttagtatc
ttttctcccgcactcattagctattaaaagaggatgtttgtttaaagctgctcagagctggt
aaactaatgttaagtcactaacggaatttaaaaggtttcattaagaactgcctgcactaga
ttcctccaccctgagacattaaacaatcacgataaacctcctgagtggtaagaacgtgtcca
tttaaaaacaggctatagattgtcatgcagttttatctactaatcggctaatgcaccgccaa
aaacaaacaaaaaaacccaaagggatgaaagtttcatccatcaaaggaaacaacagtcacct
tggttcccatcccactcatatactgccgccgtacatgtcaatcagatgaacctgtgcgtatc
tcttaacgacaattgacccacctttttaactgaagtgaagggggggttctgctccgcgaccac
ttcctggatctctcccttcaccctctgtgttctttcgggtgcaccatcgggtcaaagccgca
gcaacgccgtctctgtgtgatcgcatgtgcccttctgcacacgaccttcccccgagagtgac
cagctaccggacaggcaccaaggagggctaccgagcacctcccggaccggcggctgcaggat
cgcgagcgcctccgctagggagaccgcacgttgcgcctgtgcttcctgcggtggcgccttct
gcaaggagacctcgaccctgctccctctccggggctggatctgactccttgacggtgattcc
agacgcgagacccaaactgacggcttctagaagaggggcgagcccggccgcaagtctttcac

**Fig. 8 cont.**

**Hybrid Rhesus box of RHD negatives**

gtagctaagtcatcgttgcttccggcttcttaccgttctcccctttgtaaacggttacctcc
cgaaaacccaggctctcctccaacagtggttctcaagcgaggcgatcttccccgggaggga
tatttggcaaagtctgggggcatttttggttcactggggctgctacttgcatccactgggta
gaggcgggggatgcagctacacaacctgcgaagcacgggacagcaccctccccaacccagac
agaattagccggcccaaaacctcagtagtgcccaggctgagaaaccctgccttaaacaaaca
acaaagaaaggccaagtcccataagtgggtcaccgcgccgagactggggtccacgggacacc
ccagccacgccaagccgggaagtccccgcctcctggagctgaacccgcccctctcccagagg
tggagctgcggggggcgggaacaggcacggagaaataaacaagactaaaagtcctgagta
gcgctgtgtggccgcaaacctgaacccacctttgcaccacgcgggacccggcactcttcct
gccacccacccctgagagggctgcgcggccgaccccagtactagaaaacactcgtcacctca
ctcaagacgggtacgaaggccaacggacgccttcctttagaacgctcagcacacagagcaac
ttctcacgcctactctcaaatggcgtactccaaactagcactcccgacgtccagctgtgaac
ccagagcggcggaaagcccctgaacccagcgcccgggcatgcgcagacgcgttgttgtggtg
ggcgtggctccctccggacccggcgccccgccctccgccccgtgtccgcatgcgcgactgag
ccgggtggatggtactgctgcatccgggtgtctg
(end of Rhesus box)
gaggctgtggccgttttgttttcttggctaaaatcggggagtgaggcgggccggcgcggc
3'

**Fig. 9**

**Upstream Rhesus box of D-positives**

5' ctagaaaacactttgtcattttagaggtgtta
(start of Rhesus box)
tccaatgttcgcgcaggcactggagtcagagaaaatggagttgaatcctttctctgccactc
tttgaggagaatctcaccatttattatgcactgtagaatacaacaataaaatacagccatgt
accacataacaacatcttggtaaacaacagactgcatatatgatggtggtcatccagtaagc
taaggttaatttattattattcccttttttttttcttttttttgagatgtagtcttactctg
tcacccaggctagagtgcaatggcaccatcttggctcactgcaacctctgcctcctgggttc
aagcgaatctcctgcctcagcctcccgaagtagctgggattacaggcacccaccacatctgg
ctaatttttgtatttttagtaaagatggggtttcaccatgttggccaggctgatctcaaac
tcctgacctcaagtgatctgcctgcctcggcctcccaaagtgctgggaccataggcctgagc
cactgtgcccggccttgtttgctttttaacagttaacagtgtgctcatagaaactgctttg
acatgactgcaatcatgtgcttcatagaaacttaattagattataccactagagtcttcaga
tttttatactttttttttgaaacggagtctcactctgtcaccaggctggagtgcagtgccg
caatctcagctcgccgcaacctccgcctcccaggttcaagtgattctcctgcctcagcctcc
cgagtagctgggattacaagtgcacactaccacgcccagctaattttgcatttttactaga
cagggtttcaccatgttggctaggatagtttcaccaggatctcttggcctcatgatcagcct
gcctcggcctcccaaagtgctgggattacaggtgtgagccaccgtgcccagcctatacttcc
ctttttgaataccatttggcgttttgaagaattaacagctttgtgaacgtggcagtgcttgt
gattcaggcttccactgagaccaaggggagaacctggttgcaggacaaacagacggacagcg
tgtggcagtgtttaaatgctcttctgaaggctgatacgacagctctctgtgcactgattgca
tacgcatcccaagattatattattgtttctattgctatgtgtcacactttgccaaacagga
tgtggaaaatgaataagcggtttcttaggcacttcttaacagacaattggtcaaaatgaac
tccattgcttaagaaacacataaacaccatttagtcactgaatatagctatatgtatggttg
ctactatggggaatcttgttttgccaatttctttgaaaattctggcagaccaaggttcttt
ttgtttacacaatacttgaaaaataaaaatgaacaagccaacaaactaccaagttttcactt
acataaatgtagttacatacagaaaatgtgactgtgaatttttctaggacttttaaactat
aagcactatttgcacgaaagagaaccaatctatcaattacaaactcacataattttacagat
tttttttccctacacagcacataaaacagaaggaatttgaagccaccctccaaacacaggg
gaaggaggctgtgtgtatatcctcattgtctttcacattctaaggtggttccactcagtgac
tgaaatccttaagtgttgtattagtcggcttgggctaccataacagcagcttaaactgttgt
taagccactcagacttaaacaacagaaatttatttccttatagttctggaggctggaagttc
aaggtgccggcaaggctggtttctggtgagacctctctccctgtcttgcagatggctgcctc
ctccctgtgtcctcatagagcctgtcctctgcttttacacttctggtgtcatcttcctttttt
tttttttttttgagacagagtctcgctctatcgcccaggctggagtgcagtggcccgatcga
tctcggctcactgcaacctctgcctcccaggttcaagcaattctcctgcctcagcctcccga
gtagctgggactacaggtgcccgccatcatgtctggctaattttgtatttttagtagagac
agggtttcaccatattggccaggctggtctccaactcctgaccttgtcatctgcctgcctcg
gcctcccaaagtgctaggattacaggcgtgagccaccgcacccggcctctttctcttcttat
aaggacaccagtcctattagattagggctccaccctcatgacctcatttgaccttaactatt
atttctttaaagcacctatttccaaatatagtcactttaggggttagggcttcaaaatatga
atctgagggagatcaattcagtaaatagcagtagtcattaacggacaatatatacaaagata
atttcgtgattactgtccttatgcataaatgtcctcagtgttccactgcctttatccagatt
tactatcacaaagactttgctctgagaaaaatgtgatttctttcttttttttttttttttga
gacagagtctcactctgtcacccaggctggagtgcagtggtgcaatctcggctcactgcaat
ctccgcctcccaggttcacgccattctcttgcctcagtctcccgagtagctgggcctacagg

**Fig. 9 cont.**

**Upstream Rhesus box of D-positives**

cgcccgccaccctgcccagctaatttttgtatttttagtagagacggggtttcaccatgtt
agccaggatggtctcaatctcctgacctcgtgatccacctgcctcagcctcccaaagtgctg
ggattacaggcatgagccaccgcgcccagcagattttttttttttttttttttttgagat
ggagtcttgctgtgttgcccagcctggagtgcagtgttatgattttggctcactgcaacctc
tgtctaccatgttcaagcgattctcccacctctgcctcccgtgtagctgggatcacaggcac
acgccaccacacctagctactttttgtatttttagtagaaatggggtttcaccatgttggcc
aggatggtcccgaactcctgacctcaagtgatcctcctgcctcggcctcccaaagtgctggg
attacaggtgtgagccactgtgcctggccaaaaatgtgatttcttatttcccacattgccaa
ttccatttcaattaactataatagctatgtctattgagcactcaagtgtattctagaaactg
ttcctgattctggggatatatccatgaatcaactatagtccctgttattaagtaatctgtag
tctgactaaaccattagaaatttaaaaaatggctactttcaaagacatcttggagttcagga
gtcccacactgcgaaccatattacctaataatccaacctgcttgtaattcacttatttaacc
aatatttattgagtgccaactttgagcctaagatacagcagtaaacaaatggataaagtccc
tgtcctcatgaaacttgtattctaatggaagaaacagaaaacaaacagatataggatgtaat
atcaggtagggataaatactttgaattcaaacaaaagtatacgtagtcagggttcgccaaag
agacacagccaatcggatacatagatatataaaagagggtttatgagttagaaagggctcac
atgattacagaggctgagaagtcccacagcagattgtctgcaagctggagacccagggatac
tggtagcatggctcagtccaagtcccaaagcctcagaatcaggaaagctgatgatataattc
ttagcccaaaggccttagaaccccagcggtgacggaaaggctgatgtaggtcctggagtcct
gagacccaacagcctgggatcctgaaatccaagggcaggaatggaagcgtgtattccagctc
caagagagtaagaccaatttgcctttcttccgtttttgtttcaagccacctgcacattgagg
gcggatggttccctcttagtccattcagtcatatatcaatctcttctggaaataccctcaca
gacacactaacaaataatgcctttccagttctctaggtattctttaatccagtcaagctgac
acctaaaattaaccatcacaaaagttaaggagaaagaagacaacttgtaggggaggctgcta
tgcaagacagtgtgtgaaggaagggctctctgaagaggttaatatctgagcagagacttgaa
tgaagtgaagaagtgagccatgtgggtatggggaatacaacttccaggtagagaagacaagt
gtggtgtgtatcagggtcagcaaagaagccatgtgacagagaagggtgggccagggagagac
ggataagtgatctaactcctgaggaggtggcctggccaggagctagagcatgaagatctcgt
aggactttattctgcaaggtgaaaagccattgtattagtctgttcacaaacccgagactagg
caatttacaaaagaaagagaggtttaatggacttacagttccacatggctggggaggcctca
caatcatggcgaaaggcaatgaggagcaagtcacgtcttacgtggatggcaggcaaagacaa
agacagcttgtgcagagaaactccccttatagagccatcagatcctgttagacttattcac
tatcacaagaacagcacgggtaagacctgtccccatgattcagttacctcccactgggtccc
tcccacaacgcatgggaattcaggatgagatttgggtggggacacaaccaaaccctatcatt
ccacccatggcccctcccaaatttcatgtcctcacatttcaaaaccaatcacaccatcccaa
cagtccctcaaagtcttaaatgatttcagcattaactcaaaagtccacagtctaatgtctca
tctgagacaaggcaagtcctttccatttatgagcctataaaatccaaagcaagttagttact
tcctagatacaatggggtacaggcattgggtaaatacagccattccaaatgggataaattg
gtcaaaacaaagaggctacaggcccatgagagtccaaaatccagtggggcagtcaaatctta
aagctccaaaatgatctcctttgactccacatctcacatccaggtcacgcagatggaagggg
tgggttcccatggtcttgggcagctctgcccctgtacctttgcagggtacagcctccctctc
agctgctttcatgggctggcattgagtgtctgcagcttttccaggtacacggtgcaagctgt
cggtggatctaccattctggggtctggaggacctcttctcacagctccactaggtggtgccc
cagtagggactgtgtgtggggtctctgaccccacatttcccttctgcactgccctggcagag
gatctccatgagggccctgctcctgcagcaaacttctgactgggcatccaggcatttccgca

**Fig. 9 cont.**

Upstream Rhesus box of D-positives

catcctctttaatctaggcgaaggtttccaaaccccaattcttgacttctgtgcactcgcag
tctcaacaccacatggaagctgtcaaggcttggggcttgcactccccgaagctacagcccaa
gctctaccttgcctcccgtcagtcatggttgggagtggctgggatgcagggcaccaagtccc
taggctgcacacagcatgaggaccccgggcctggccaacaaaccatttttcctgatacct
ctggacctgtgatgggaggggttgccataaagacctctgacatgccctggagacattttccc
cattgtcttgggaattagcatttggctcctgttactcatgcaatttctgcagccagcttga
atttctcctcagaaaatgggaattttctttctatcacattgtcaggctgcaaattttccg
aacttttatgctctgcttccccttataaaactgaatgtctttaacagcacccaagtcacctct
tgaatgctttgctgcttagaaatttctcctgccagatactctaaatcatctctctgaagttc
aaagttctacaaatatctcgtgcaggggcaaaatgccgccagtatctttgctaaaacataac
aagagtccccttgctccagttcccaacaagttcctcatttccgtctgagaccacctcagcc
tatggactttattgtccacagtgctatcagcattttggcaaagccattcaacaagtctcta
ggaagttccaaactttcccacatttgcctgtcttcttctgagccctccaaactgttccaaac
cctgcctgttacccagttccaaagtcacatacccatttttgagtatctacggcagcacccca
ctctactggtaccaatttagccactgaagtagttggagaacagaagtaatagactctggttt
acattgtaaaagcttctctgtggctgctgtgtgaagaaaatatatgagaatgaagccccaag
atgaagcagggacacagttgcagtggttagagtaagaaatgctgctggctggcactgaagtg
atagcctggaggtttgtgtgtgcacatgcatgtgtatgtgttttacgatagtaggcccaaca
gatactgtaatccacacttgttttttttttgagacagagtctcacctgttgcctagacta
gaatgcagtggcacaatcttggctcactacaacctccacctcccaggttcaaacaatccttg
tgcttcagcctcccgagtagttgggattacaggtgtgtgccaccgtgcccagctatatttttt
tgtatttttagcagagatgggatttgccacattggccaggctggtcttgaactcctggcct
caagcaatcctcccaccttagcctcccaaagtgctgagccaccacacctggccgcaactgat
ttttaatcatgaaatgacacatacatttaaaaaacccaatacctataatattcctggctagt
actcttcacatctatatcatcaaaaacaaagaaagtatgtgaaactgacacagccaagggga
gactaaggagacataacaattaactgtaatgtggtattctggaggggatcctggaacagaaa
aagacattaggcaaaaaactaaagaaatctgaataaaatgtggatgtcagttaataataatg
tatcatattagtccagtaattgtaacaaatataccacaataatgaaagccattaattatagg
gaaaatggaggggttaatatgggtggctggcttttgctatttctagcagctccattttatct
gcaaaagacaaacattcattaagtcccaaaaaggtaaagaatgacaaattaagcatgtatct
tattagtaagagtaatataaagatgctcactcctatttataaatatttgacaatcatgttaa
ggccacaaaagagaaaaaagggtaggggcaaaaaacgcaaagagaaaggagttagtatcttt
tctcccgcactcattagctattaaaagaggatgtttgtttaaagctgctcagagctggtaaa
ctaatgttaagtcactaacgggaatttaaaaggtttcattaagaactgcctgcactagattc
ctccaccctgagacattaaacaatcacgataaacctcctgagtggtaagaacttgtccattt
aaaaacaggctatagattgtatcatgcagttttatctactaatcggctaatatcccgccaaa
aacaaaaaaccccaaagggatgaaagtttcatccatcaaaggaaacaacagtcaccttggtt
cccatctcactcatatactgccgccgtacatgtcaatcagatgaacctgtgcgtatctctta
atgacaattgacccacatttttgactgaagtgaaaggggggttctgctccgcgaccacttcct
ggatctcccccctccaccctctgtgttctttcgggtgcaccatcgggtcaaagccgcagcaac
gccgtctctgtgtgatcgcatgtgcccttctgcacacgaccttcccccgagagtgaccagct
accggacaggcaccaaggagggctaccgagcacctcccggaccggcggctgcaggatcgcga
gcgcctccgctagggagactgcacgttgcgcctgtgcttcctgcggtggcgccttctgcaag
gagacctcgaccctgctccctctccggggctggatctgactccttgacggtgattccagacg
cgagacccaaactgacggcttctagaagaggggcgagcccggccgcaagtctttcacgtagc

**Fig. 9 cont.**

Upstream Rhesus box of D-positives

```
taagtcatcgttgcttccggcttcttaccgttctcccctttgtaaacggttacctcccgaaa
acccaggctctcctccaacagtggttctcaagcgaggcgatcttccccgggaggggatattt
ggcaaagtctgggggcatttttggttcactggggctgctacttgcatccactgggtagaggc
ggggatgcagctacacaacctgcgaagcacgggacagcaccctccccaacccagacagaat
tagccggcccaaaacctcagtagtgcccaggctgagaaaccctgccttaaacaaacaacaaa
gaaaagccaagtcccataagtgggtcaccgcgccgagactggggtccacgggacaccccagc
cacgccaagccgggaagtccccgcctcctggagctgaacccgcccctctcccagaggtggag
ctgcggggggcgggaacaggcacggagaaaataaacaagactaaaaagtcctgagtagcgct
gtgtggccgcaaacctgaacccacctttgcaccacgcgggacccggcacgcttcctgccac
ccacccctgagagggctgcgcggccgaccccagtactagaaaacactcgtcacctcaatcaa
gacgggtacgaaggccaacggacgccttcctttagaacgctcagcacacagagcaacttctc
acgcctactctcaaatggcgtactccaaactagcactcccgacgtccagctgtgaacccaga
gcggcggaaagcccctgaacccagcgcccgggcatgcgcagacgcgttgttgtggtgggcgt
ggctccctccggacccggcgccccgccctccgccccgtgtccgcatgcgcgactgagccgcg
ggggtggtactgctgcatccgggtgtctg
(end of Rhesus box)
aagatccgatgaaataacatatgcaaaatgattgggtccgtgattggcattccagaaatgg
3'
```

**Fig. 10**

**Upstream Rhesus box of D-positives**

5' ctagaaaacactttgtcattttagaggtgtta
(start of Rhesus box)
tccaatgttcgcgcaggcactggagtcagagaaatggagttgaatcctttctctgccactc
tttgaggagaatctcaccatttattatgcactgtagaatacaacaataaaatacagccatgt
accacataacaacatcttggtaaacaacagactgcatatatgatggtggtcatccagtaagc
taaggttaatttattattattcccttttttttttctttttttgagatgtagtcttactctg
tcacccaggctagagtgcaatggcaccatcttggctcactgcaacctctgcctcctgggttc
aagcgaatctcctgcctcagcctccgaagtagctgggaattacaggcacccaccacatctgg
ctaatttttgtatttttagtaaagatggggtttcaccatgttggccaggctgatctcaaac
tcctgacctcaagtgatctgcctgcctcggcctcccaaagtgctgggaccataggcctgagc
cactgtgcccggccttgtttgctttttaacagttaacagtgtgctcatagaaactgctttg
acatgactgcaatcatgtgcttcatagaaacttaattagattataccactagagtcttcaga
ttttatactttttttttgaaacggagtctcactctgtcaccaggctggagtgcagtgccg
caatctcagctcgccgcaacctccgcctcccaggttcaagtgattctcctgcctcagcctcc
cgagtagctgggattacaagtgcacactaccacgcccagctaattttgcatttttactaga
cagggtttcaccatgttggctaggatagtttcaccaggatctcttggcctcatgatcagcct
gcctcggcctcccaaagtgctgggattacaggtgtgagccaccgtgcccagcctatacttcc
ctttttgaataccatttggcgttttgaagaattaacagctttgtgaacgtggcagtgcttgt
gattcaggcttccactgagaccaaggggagaacctggttgcaggacaaacagacggacagcg
tgtggcagtgtttaaatgctcttctgaaggctgatacgacagctctctgtgcactgattgca
tacgcatcccaagattatattattgttttctattgctatgtgtcacactttgccaaacagga
tgtggaaaatgaataagcggtttcttaggcacttcttaacagacaattggtcaaaatgaac
tccattgcttaagaaacacataaacaccatttagtcactgaatatagctatatgtatggttg
ctactatggggaatcttgttttgccaatttctttgaaaattctggcagaccaaggttcttt
ttgtttacacaatacttgaaaaataaaaatgaacaagccaacaaactaccaagttttcactt
acataaatgtagttacatacagaaaatgtgactgtgaatttttctaggactttaaactat
aagcactatttgcacgaaagagaaccaatctatcaattacaaactcacataattttacagat
ttttttttccctacacagcacataaaacagaaggaatttgaagccaccctccaaacacaggg
gaaggaggctgtgtgtatatcctcattgtctttcacattctaaggtggttccactcagtgac
tgaaatccttaagtgttgtattagtcggcttgggctaccataacagcagcttaaactgttgt
taagccactcagacttaaacaacagaaatttatttccttatagttctggaggctggaagttc
aaggtgccggcaaggctggtttctggtgagacctctctccctgtcttgcagatggctgcctc
ctccctgtgtcctcatagagcctgtcctctgcttttacacttctggtgtcatcttcctttttt
tttttttttttgagacagagtctcgctctatcgcccaggctggagtgcagtggcccgatcga
tctcggctcactgcaacctctgcctcccaggttcaagcaattctcctgcctcagcctcccga
gtagctgggactacaggtgcccgccatcatgtctggctaattttgtatttttagtagagac
agggtttcaccatattggccaggctggtctccaactcctgaccttgtcatctgcctgcctcg
gcctcccaaagtgctaggattacaggcgtgagccaccgcacccggcctctttctcttcttat
aaggacaccagtcctattagattagggctccaccctcatgacctcatttgaccttaactatt
atttctttaaagcacctatttccaaatatagtcactttaggggttagggcttcaaaatatga
atctgagggagatcaattcagtaaatagcagtagtcattaacggacaatatatacaaagata
atttcgtgattactgtccttatgcataaatgtcctcagtgttccactgcctttatccagatt
tactatcacaaagactttgctctgagaaaatgtgatttctttctttttttttttttttga
gacagagtctcactctgtcacccaggctggagtgcagtggtgcaatctcggctcactgcaat
ctccgcctcccaggttcacgccattctcttgcctcagtctcccgagtagctgggcctacagg

**Fig. 10 cont.**

Upstream Rhesus box of D-positives

cgcccgccaccctgcccagctaatttttgtatttttagtagagacggggtttcaccatgtt
agccaggatggtctcaatctcctgacctcgtgatccacctgcctcagcctcccaaagtgctg
ggattacaggcatgagccaccgcgcccagcagattttttttttttttttttttgagat
ggagtcttgctgtgttgcccagcctggagtgcagtgttatgatttggctcactgcaacctc
tgtctaccatgttcaagcgattctcccacctctgcctcccgtgtagctgggatcacaggcac
acgccaccacacctagctactttttgtatttttagtagaaatggggtttcaccatgttggcc
aggatggtcccgaactcctgacctcaagtgatcctcctgcctcggcctcccaaagtgctggg
attacaggtgtgagccactgtgcctggccaaaaatgtgatttcttatttcccacattgccaa
ttccatttcaattaactataatagctatgtctattgagcactcaagtgtattctagaaactg
ttcctgattctggggatatatccatgaatcaactatagtccctgttattaagtaatctgtag
tctgactaaaccattagaaatttaaaaaatggctactttcaaagacatcttggagttcagga
gtcccacactgcgaaccatattacctaataatccaacctgcttgtaattcacttatttaacc
aatatttattgagtgccaactttgagcctaagatacagcagtaaacaaatggataaagtccc
tgtcctcatgaaacttgtattctaatggaagaaacagaaaacaaacagatataggatgtaat
atcaggtagggataaatactttgaattcaaacaaaagtatacgtagtcagggttcgccaaag
agacacagccaatcggatacatagatatataaaagagggtttatgagttagaaaggctcac
atgattacagaggctgagaagtcccacagcagattgtctgcaagctggagacccaggatac
tggtagcatggctcagtccaagtcccaaagcctcagaatcaggaaagctgatgatataattc
ttagcccaaaggccttagaaccccagcggtgacggaaaggctgatgtaggtcctggagtcct
gagacccaacagcctgggatcctgaaatccaagggcaggaatggaagcgtgtattccagctc
caagagagtaagaccaatttgcctttcttccgtttttgtttcaagccacctgcacattgagg
gcggatggttccctcttagtccattcagtcatatatcaatctcttctggaaataccctcaca
gacacactaacaaataatgcctttccagttctctaggtattctttaatccagtcaagctgac
acctaaaattaaccatcacaaaagttaaggagaaagaagacaacttgtaggggaggctgcta
tgcaagacagtgtgtgaaggaagggctctctgaagaggttaatatctgagcagagacttgaa
tgaagtgaagaagtgagccatgtgggtatggggaatacaacttccaggtagagaagacaagt
gtggtgtgtatcagggtcagcaaagaagccatgtgacagagaagggtgggccagggagagac
ggataagtgatctaactcctgaggaggtggcctggccaggagctagagcatgaagatctcgt
aggactttattctgcaaggtgaaaagccattgtattagtctgttcacaaacccgagactagg
caatttacaaaagaaagagaggtttaatggacttacagttccacatggctggggaggcctca
caatcatggcgaaaggcaatgaggagcaagtcacgtcttacgtggatggcaggcaaagacaa
agacagcttgtgcagagaaactccccttatagagccatcagatcctgttagacttattcac
tatcacaagaacagcacgggtaagacctgtccccatgattcagttacctcccactgggtccc
tcccacaacgcatgggaattcaggatgagatttgggtggggacacaaccaaaccctatcatt
ccacccatggcccctcccaaatttcatgtcctcacatttcaaaaccaatcacaccatcccaa
cagtccctcaaagtcttaaatgatttcagcattaactcaaaagtccacagtctaatgtctca
tctgagacaaggcaagtcctttccatttatgagcctataaaatccaaagcaagttagttact
tcctagatacaatgggggtacaggcattgggtaaatacagccattccaaatgggataaattg
gtcaaaacaaagaggctacaggcccatgagagtccaaaatccagtggggcagtcaaatctta
aagctccaaaatgatctcctttgactccacatctcacatccaggtcacgcagatggaagggg
tgggttcccatggtcttgggcagctctgcccctgtacctttgcagggtacagcctccctctc
agctgctttcatgggctggcattgagtgtctgcagcttttccaggtacacggtgcaagctgt
cggtggatctaccattctggggtctggaggacctcttctcacagctccactaggtggtgccc
cagtagggactgtgtgtggggtctctgaccccacatttcccttctgcactgccctggcagag

**Fig. 10 cont.**

Upstream Rhesus box of D-positives

gatctccatgagggccctgctcctgcagcaaacttctgactgggcatccaggcatttccgca
catcctctttaatctaggcgaaggtttccaaaccccaattcttgacttctgtgcactcgcag
tctcaacaccacatggaagctgtcaaggcttggggcttgcactccccgaagctacagcccaa
gctctaccttgcctcccgtcagtcatggttgggagtggctgggatgcagggcaccaagtccc
taggctgcacacagcatgaggaccccgggcctggccaacaaaaccatttttcctgatacct
ctggacctgtgatgggaggggttgccataaagacctctgacatgccctggagacattttccc
cattgtcttgggaattagcatttggctcctgttactcatgcaaatttctgcagccagcttga
atttctcctcagaaaatgggaatttttcttttctatcacattgtcaggctgcaaattttccg
aacttttatgctctgcttcccttataaaactgaatgtctttaacagcacccaagtcacctct
tgaatgctttgctgcttagaaatttctcctgccagatactctaaatcatctctctgaagttc
aaagttctacaaatatctcgtgcaggggcaaaatgccgccagtatctttgctaaaacataac
aagagtccccttttgctccagttcccaacaagttcctcatttccgtctgagaccacctcagcc
tatggactttattgtccacagtgctatcagcattttgggcaaagccattcaacaagtctcta
ggaagttccaaactttcccacatttgcctgtcttcttctgagccctccaaactgttccaaac
cctgcctgttacccagttccaaagtcacatacccattttgagtatctacggcagcacccca
ctctactggtaccaatttagccactgaagtagttggagaacagaagtaatagactctggttt
acattgtaaaagcttctctgtggctgctgtgtgaagaaaatatatgagaatgaagccccaag
atgaagcagggacacagttgcagtggttagagtaagaaatgctgctggctggcactgaagtg
atagcctggaggtttgtgtgtgcacatgcatgtgtatgtgttttacgatagtaggcccaaca
gatactgtaatccacacttgtttttttttttgagacagagtctcacctgttgcctagacta
gaatgcagtggcacaatcttggctcactacaacctccacctcccaggttcaaacaatccttg
tgcttcagcctcccgagtagttgggattacaggtgtgtgccaccgtgcccagctatattttt
tgtatttttagcagagatgggatttgccacattggccaggctggtcttgaactcctggcct
caagcaatcctcccaccttagcctcccaaagtgctgagccaccacacctggccgcaactgat
ttttaatcatgaaatgacacatacatttaaaaaacccaatacctataatattcctggctagt
actcttcacatctatatcatcaaaaacaaagaaagtatgtgaaactgacacagccaagggga
gactaaggagacataacaattaactgtaatgtggtattctggaggggatcctggaacagaaa
aagacattaggcaaaaaactaaagaaatctgaataaaatgtggatgtcagttaataataatg
tatcatattagtccagtaattgtaacaaatataccacaataatgaaagccattaattatagg
gaaaatggaggggttaatatgggtggctggcttttgctatttctagcagctccattttatct
gcaaaagacaaacattcattaagtcccaaaaaggtaaagaatgacaaattaagcatgtatct
tattagtaagagtaatataaagatgctcactcctatttataaatatttgacaatcatgttaa
ggccacaaaagagaaaaaagggtaggggcaaaaaacgcaaagagaaaggagttagtatcttt
tctcccgcactcattagctattaaaagaggatgtttgtttaaagctgctcagagctggtaaa
ctaatgttaagtcactaacgggaatttaaaaggtttcattaagaactgcctgcactagattc
ctccaccctgagacattaaacaatcacgataaacctcctgagtggtaagaacttgtccattt
aaaaacaggctatagattgtatcatgcagttttatctactaatcggctaatatcccgccaaa
aacaaaaaaccccaaagggatgaaagtttcatccatcaaaggaaacaacagtcaccttggtt
cccatctcactcatatactgccgccgtacatgtcaatcagatgaacctgtgcgtatctctta
atgacaattgacccacattttgactgaagtgaaaggggttctgctccgcgaccacttcct
ggatctcccccctccaccctctgtgttctttcgggtgcaccatcgggtcaaagccgcagcaac
gccgtctctgtgtgatcgcatgtgcccttctgcacacgaccttcccccgagagtgaccagct
accggacaggcaccaaggagggctaccgagcacctcccggaccggcggctgcaggatcgcga
gcgcctccgctagggagactgcacgttgcgcctgtgcttcctgcggtggcgccttctgcaag

**Fig. 10 cont.**

Upstream Rhesus box of D-positives

gagacctcgaccctgctccctctccggggctggatctgactccttgacggtgattccagacg
cgagacccaaactgacggcttctagaagaggggcgagcccggccgcaagtctttcacgtagc
taagtcatcgttgcttccggcttcttaccgttctcccctttgtaaacggttacctcccgaaa
acccaggctctcctccaacagtggttctcaagcgaggcgatcttccccgggaggggatattt
ggcaaagtctgggggcatttttggttcactggggctgctacttgcatccactgggtagaggc
gggggatgcagctacacaacctgcgaagcacgggacagcaccctccccaacccagacagaat
tagccggcccaaaacctcagtagtgcccaggctgagaaaccctgccttaaacaaacaacaaa
gaaaagccaagtcccataagtgggtcaccgcgccgagactggggtccacgggacaccccagc
cacgccaagccgggaagtccccgcctcctggagctgaacccgcccctctcccagaggtggag
ctgcgggggcgggaacaggcacggagaaaataaacaagactaaaaagtcctgagtagcgct
gtgtggccgcaaacctgaacccacctttgcaccacgcgggacccggcacgcttcctgccac
ccacccctgagagggctgcgcggccgaccccagtactagaaacactcgtcacctcaatcaa
gacgggtacgaaggccaacggacgccttcctttagaacgctcagcacacagagcaacttctc
acgcctactctcaaatggcgtactccaaactagcactcccgacgtccagctgtgaacccaga
gcggcggaaagcccctgaacccagcgcccgggcatgcgcagacgcgttgttgtggtgggcgt
ggctccctccggacccggcgccccgccctccgccccgtgtccgcatgcgcgactgagccgcg
ggggtggtactgctgcatccgggtgtctg
(end of Rhesus box)
aagatccgatgaaataacatatgcaaaatgattgggtccgtgattggcattccagaaatgg
3'

**Fig. 11**

```
ctgatctaca taggaattgt tttcaagaca tttctgcatt cctctagtga cagggtgctc      60
actacctcat gagtatttca gtggacaact gtaatggtca ataaagtatc cactttccac     120
ctccctgcag ctcctggccc tggctttatt ctctggggct ccacacattc agtttacact     180
cagtggccag tggctgggac cattgtagaa aataaggaaa ctccaattcc ttccttcttt     240
tcttcctctt tcatctcttc ctccctctct acatccctct ctctcttcct tccttcctcg     300
acacttacca tgtaccagac cttctgccag gcacatggat gggagcacag gggaagttgg     360
ctgcagggtt agaactaagt cccaagcccc ctaaagctca tgccagggga ctggactgtc     420
cagtactgag ggatggggat gctgaggctg gtggccttcc tcaaatgcac tgtagtgccc     480
caggcagagt cctgggctgc cctgtgagga ggtgaccaga ggtagagcaa cttcacccta     540
aggctggatc aggatcccct ccaggttttt actagagcca aacccacatc tcctttctct     600
tctgccaccc ccccttaaaa tgcttagaaa cacatagatt taaatacaaa ttcaaatgta     660
agtaatttca actgtgtaac tatgaggagt cagttctacg tgggtcctat ctgtatcctc     720
cccagggctc agctccattc tttgctttca ttcattctca ttcaatacat tgttgttaag     780
agctcactgg gtgccctctc tgtcatgtag taaggtttta aaaagaaagc ctcttctgag     840
cttcagtttc cttattcata aaataggagt attgatccgt tccttgcttt tcttacaagg     900
atatgctgaa gatgactgaa gtacagagta aagaaggatt atgtttgggt gtcaaaggaa     960
tagaatgccc tctttcaaac tgagcacagc aggaacctgt aacaggaaca cagcaacttg    1020
ttgaatgaat gacaatattg gaaaacatac atttcctccc ctccccatca tagtccctct    1080
gcttccgtgt taactccata gagaggccag cacaaccagc cttgcagcct gagataaggc    1140
ctttggcggg tgtctcccct atcgctccct caagccctca agtaggtgtt ggagagaggg    1200
gtgatgcctg gtgctggtgg aacccctgca cagagacgga cacaggatg                1249
```

**Fig. 12**

2938 CTAGAGAGGGAAGTTTTTGAAAATTAAACACTGTCTAATTTTCTGCAAAGTTTTTATTCATGAATTAAGAGTATTTCCCTTAGTCCATTATTCCCAAGGC *RHCE*
CTAGAGAGGGAAGTTTTTGAAAATTAAACACTGTCTAATTTTCTGCAAAGTTTTTATTCATGAATTAAGAGTATTTCCCTTTGTCCATTATTCCCAAGGC *$Cde^s$*
CTAGAGAGGGAAGTTTTTGAAAATTAAACACTGTCTAATTTTCTGCAAAGTTTTTATTCATGAATTAAGAGTATTTCCCTTTGTCCATTATTCCCAAGGC *RHD*

|*********************** breakpoint region ****************************************
3038 AAATATGGAAGTTTGATCATATGCTAATCATACTAAAGCTGGATTCTCTTTAAGAGATTGAGAAATTAAAAGGCAAAAGCTGATATATCATGTTTAGTTA *RHCE*
AAATATGGAAATTTGATCATGTACTAATCATACTAAAGCTGGATTCTCTTTAAGAGATTGAGAAATTAAAAGGCAAAAGCTGATATGTCATGTTTAGTTA *$Cde^s$*
AAATATGGAAATTTGATCATGTACTAATCATAATAAAGCTGGATTCTCTTTAAGAGATTGAGAAATTAAAAGGCAAAAGCTGATATATCATGTTTAGTTA *RHD*

************************************* breakpoint region **********************|
3138 TACTGTGAGTCTTATAAGAAGCTGGGAGGCAACCCCATTAACTCACCAGAATACAGAACTCAGTCTCACAACTTAAATATAATTCCTCTCAAACCTTTTC *RHCE*
TATTGTGAGTCTTATAAGAAGCTGGGAGGCAACCCCATTAACTCACCAGAATACAGAACTCAGTCTCACAACTTAAATATAATTCCTCTCAAACCTTTTC *$Cde^s$*
TATTGTGAGTCTTATAAGAAGCTGGGAGGCAACCCCATTAACTCACCAGAATACAGAACTCAGTCTCACAACTTAGATATAATTCCTCTCAAACCTTTTC *RHD*

3238 CTCAAAG-TTAAATTCTGAAAATAATCTTGTGATTAAGAGAAGAAGGCTGTCCACCAATGGACTTATCTGTTATTTCTTCCTTATTGTGAGCTTAATGGC *RHCE*
CTCAAAG-TTAAATTCTGAAAATAATCTTGTGATTAAGAGAAGAAGGCTGTCCACCAATGGACTTATCTGTTATTTCTTCCTTATTGTGAGCTTAATGGC *$Cde^s$*
CTCAAAGATTAAATTCTGAAAATAATCTTGTGATTAAGAGAAGAAGGCTGTCCACCAATGGGCTTATCTGTTATTTCTTCCTTATTGTGAGCTTAATGGC *RHD*

3337 ATGACAAAGCAGAGGCAAAGAGGCATACATCAATTCTTCAAAGTAGGAAGTCAAAAAGGTCAGAGCTTCCACAGCATGGCAACAGCTTTGCAGATGCCCA *RHCE*
ATGACAAAGCAGAGGCAAAGAGGCATACATCAATTCTTCAAAGTAGGAAGTCAAAAAGGTCAGAGCTTCCACAGCATGGCAACAGCTTTGCAGATGCCCA *$Cde^s$*
ATGACAAAGCAGAGGCAAAGAGGCATACATCAATTCTTCAAAGTAGGAAGTCAAAAAGGTCAGAGCTTCCACAGCATGGCAACAGCTTTGCAGATGCCCA *RHD*

3437 CATCGTGATAGTTGAAATAGCAAAGCCCAGCAAAGGTTAAAGCTGAAAATGCCAAAAGCCCTGCCTTGGCAGCTTTCTGCGAGGCATCCCCATGAACATA *RHCE*
CATCGTGATAGTTGAAATAGCAAAGCCCAGCAAAGGTTAAAGCTGAAAATGCCAAAAGCCCTGCCTTGGCAGCTTTCTGCGAGGCATCCCCATGAACATA *$Cde^s$*
CATCGTGATAGTTGAAATAGCAAAGCCCAGCAAAGGTTAAAGCTGAAAATGCCAAAAGCCCTGCCTTGGCAGCTTTCTGCGAGGCATCCCCATGAACATA *RHD*

3537 GTCAGTAACAACTTGTCCAAGGCCCCAGTGACCATGAAGAGTGAGGGCTGCAGCCAGGGAATAGTCCGTCGCAGAGCAAGGATTCAAATAAGCAGCCGGA *RHCE*
GTCAGTAACAACTTGTCCAAGGCCCCAGTGACCATGAAGAGTGAGGGCTGCAGCCAGGGAATAGTCCGTCGCAGAGCAAGGATTCAAATAAGCAGCCGGA *$Cde^s$*
ATCAGTAACAACTTGTTCAAGGCCCCAGTGACCATGAAGAGTGAGGGCTGCAGCC-GGGAATAGTCCGTCGCAGAGCAAGGATTCAAATAAGCAGCCGGA *RHD*

**Fig. 13**

```
2726 TTGTATCTCTTTTTACAGCTACCTCCCATTTCCCTTCTATTTCAAGCTAGTAACACAGTTTTCTTTTAAATTCATTTATTTAAATGTAAAAATAAGTCTA RHCE
     TTGTATCTCTTTTTACAGCTACCTCCCATTTCCCTTCTATTTCAAGCTAGTAACACAGTTTTCTTTTAAATTCATTTATTTAAATGTAAAAATAAGTCTA Cde^s
     TTGTATCTCTTTTTACAGCTACCTCCCATTTCCCTTCTATTTCAAGCTAGTAACTCAGTTTTCTTTTAAATTCAATTATTTAAATGTAAAAATAAGTCTA RHD

2826 TTTGGAGAAAAAAAAATTTTTAATAGCATCTCTGGAATGCCAGTATGGCTAAATTCATGAATGTTGTCCTCAAATGCTGAAATCTGGGAAGCATCTGGCCA RHCE
     TTTGGAGAAAAAAAAATTTTTAATAGCATCTCTGGAAGGCCAGTATGGCTAAATTCATGAATGTTGTCCTCAAATGCTGAAATCTGGGAAGCATCTGGCCA Cde^s
     TTTGGAGAAAAAAAAATTTT-AATAGCATCTCTGGAATGCCAGTATGGCTAAATTCATGAATGTTGTCCTCAAATGCTGAAATCTGGGAAGCATCTGGCCA RHD

                                                    |*************** breakpoint region *****************
2926 AGCTTTGTGGACAGGCCTGCCTAGTTTGAATCCCAAGAGCCACTCATTCCGAGCCACAAAACATTGGAATTCTTGGTTCACTTCCCTAACCTGAACTTGT RHCE
     AGCTTTGTGGACAGGCCTGCCTAGTTTGAATCCCAAGAGCCACTCATTCCGAGCCACAAAACATTGGAATTCTTGGTTCACTTCCCTAACCTGAACTTGC Cde^s
     AGCTTTGTGGACAGGCCTGCCTAGTTTGAATCCCAAGAGCCACCCAGTCCAAGCCACAAAACATTGGAATTCTTGGTTCACTTCCCTAACCTGAACTTGC RHD

     ************************************ breakpoint region ***********************************************
3026 CCTCTGTGAAATAGGGACATTAATAGCTCACTCACAGGGCTGCTGTGAGGACATGTGTTGAGCTGAGGGTCTCGCCAGGGGAGACCCTGTGCAGGGAGAC RHCE
     CCTCTGTGAAATAGGGACATTAATAGCTCACTCACAGGGCTGCTGTGAGGACATGTGTTGAGCTGAGGGTCTGGTCAGGGGAGACCCTGTGCAGGGAGAC Cde^s
     CCTCTGTGAAATAGGGACACTAATAGCTCACTCACAGGGCTGCTGTGAGGACATGTGTTGAGCTGAGGGTCTCGCCAGGGGAGACCCTGTGCAGGGAGAC RHD

     ************************************ breakpoint region ***********************************************
3126 TGTTATCATGGTGATGGATTTCTGCTTCATTCATTTCTTTTTCCAGACAGCATCATATAGAATGAGTTGTGGGGTGGCAGTCAGCAGGTTTGGGTTTATC RHCE
     TGTTATCATGGTGATGGATTTCTGCTTCATTCATTTCTTTTTCCAGACAGCATCATATAGAATGAGTTGTGGGGTGGCAGTCAGCAGGTTTGGGTTTATC Cde^s
     TGTTATCATGGTGATGGATTTCTGCTTCATTCATTTCTTTTTCCAGACAGCATCATATAGAATGAGTTGTGGGGTGGCAGTCAGCAGGTTTGGGTTTATC RHD

     ************************************ breakpoint region ***********************************************
3226 CTCTATTCTGCCACTTATTACTTAAAAAAA------AAAACCCAACTTATATAGTATAAGCTATATCCAGAAAAGTGCAAATATCATACAAGTACCATTT RHCE
     CTCTATTCTGCCACTTATTACTTAAAAAAACCCCAAAAAACCCAACTTATATAGTATAAGCTATATCCAGAAAAGTGCAAATATCATACAAGTACCATTT Cde^s
     CTCTATTCTGCCACTTATTACTTAAAAAAACCCCAAAAAACCCAACTTATATAGTATAAGCTATATCCAGAAAAGTGCAAATATCATACAAGTACCATTT RHD
```

**Fig. 13 cont.**

```
     ************************************ breakpoint region ***************************************************
3320 GATGAATCTTCTGATATCCCCACATAACCAACACCCAGAACCTCTTCTTGTCTCATTCCAGGATAACCACTAACCTGACTTCTAACAGCATCAGTCAGTT RHCE
     GATGAATCTTCTGATATCCCCACATAACCAACACCCAGAACCTCTTCTTGTCTCATTCCAGGATAACCACTAACCTGACTTCTAACAGCATCAGTCAGTT Cde^s
     GATGAATCTTCTGATATCCCCACATAACCAACACCCAGAACCTCTTCTTGTCTCATTCCAGGATAACCACTAACCTGACTTCTAACAGCATCAGTCAGTT RHD

     ************************************ breakpoint region ***************************************************
3420 TTGTCTGTTTTTGTACATTATATATGTGATGGTTTGAATGTGTCCCCCAAATTTCATGTGCTAGAAACTTAATCCTTCAATTCATATGTTGATGCTATTT RHCE
     TTGTCTGTTTTTGTACATTATATATGTGATGGTTTGAATGTGTCCCCCAAATTTCATGTGCTGGAAACTTAATCCTTCAATTCATATGTTGATGCTATTT Cde^s
     TTGTCTGTTTTTGTACATTATATATGTGATGGTTTGAATGTGTCCCCCAAATTTCATGTGCTGGAAACTTAATCCTTCAATTCATATGTTGATGGTTTTT RHD

     ************************************ breakpoint region ***************************************************
3520 GGAGGAAGGGCCTTTGGGAAGTAATTAGGATTAGATAAGGTCATGGGGTGAGGTATGATGGCACTGGTGACTTATAAGAAGAGAAAGAGAAATCTGAGCT RHCE
     GGAGGAAGGGCCTTTGGGAAGTAATTAGGATTAGATAAGGTCATGGGGTGAGGTATGATGGCACTGGTGACTTATAAGAAGAGAAAGAGAAATCTGAGCT Cde^s
     GGAGGAAGGGCCTTTGGGAAGTAATTAGGATTAGATAAGGTCATGGGGTGAGGTATGATGGCACTGGTGACTTATAAGAAGAGAAAGAGAAATCTGAGCT RHD

     * breakpoint region *|
3620 GGCATGCTCTTGCCCTCTCACCGTGTGATGACTTCTCCATGTCATGATGCAGCAAGAAGGCCCTCACCAGATGGTGGCACCATGCTTTTGGACTTCCCAG RHCE
     GGCATGCTCTTGCCCTCTCACTGTGTGATGACTTCTCCATGTCATGATGCAGCAAGAAGGCCCTCACCAGATGGTGGCACCATGCTTTTGGACTTCCCAG Cde^s
     GGCATGCTCTTGCCCTCTCACTGTGTGATGACTTCTCCATGTCATGATGCAGCAAGAAGGCCCTCACCAGATGGTGGCACCATGCTTTTGGACTTCCCAG RHD
```

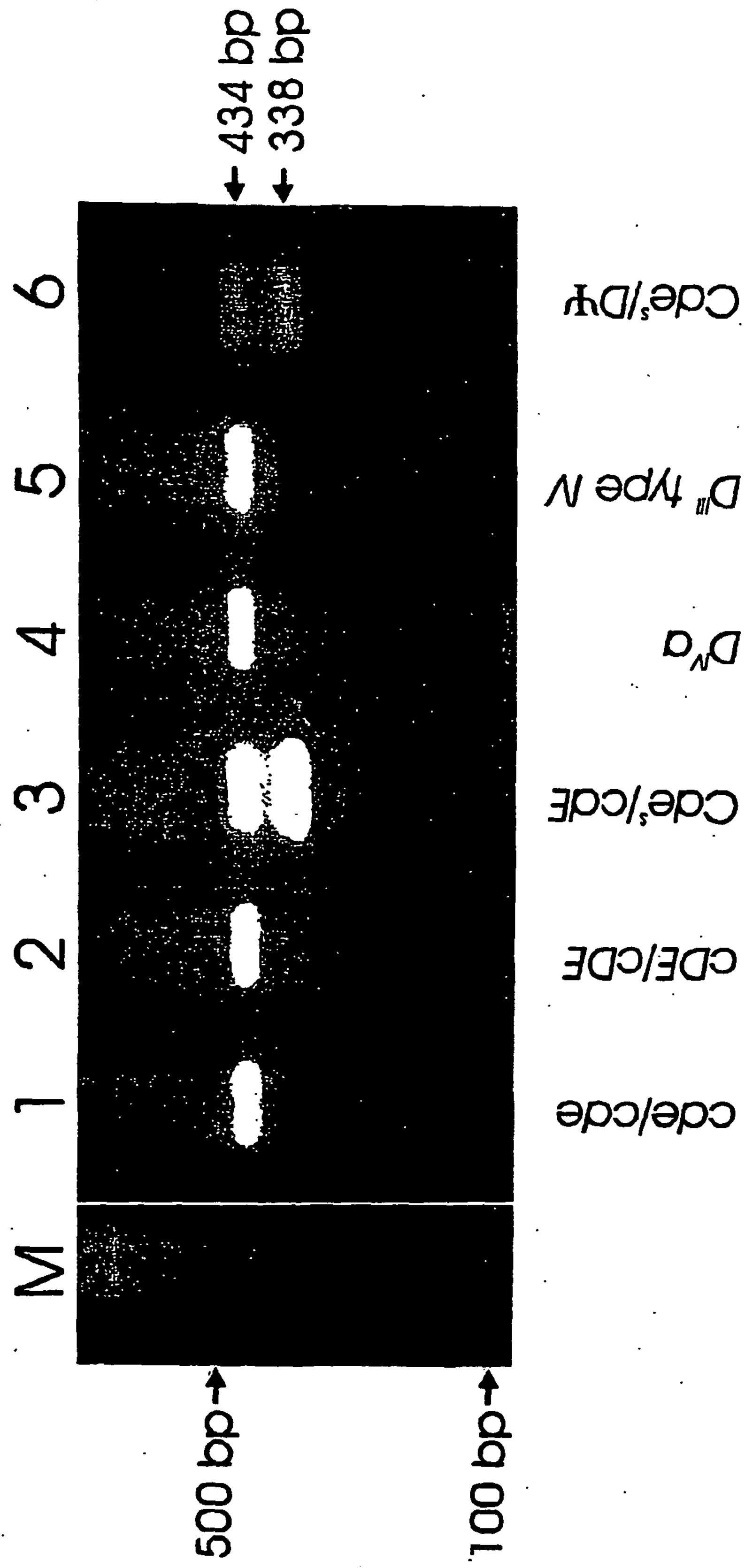



Fig. 14

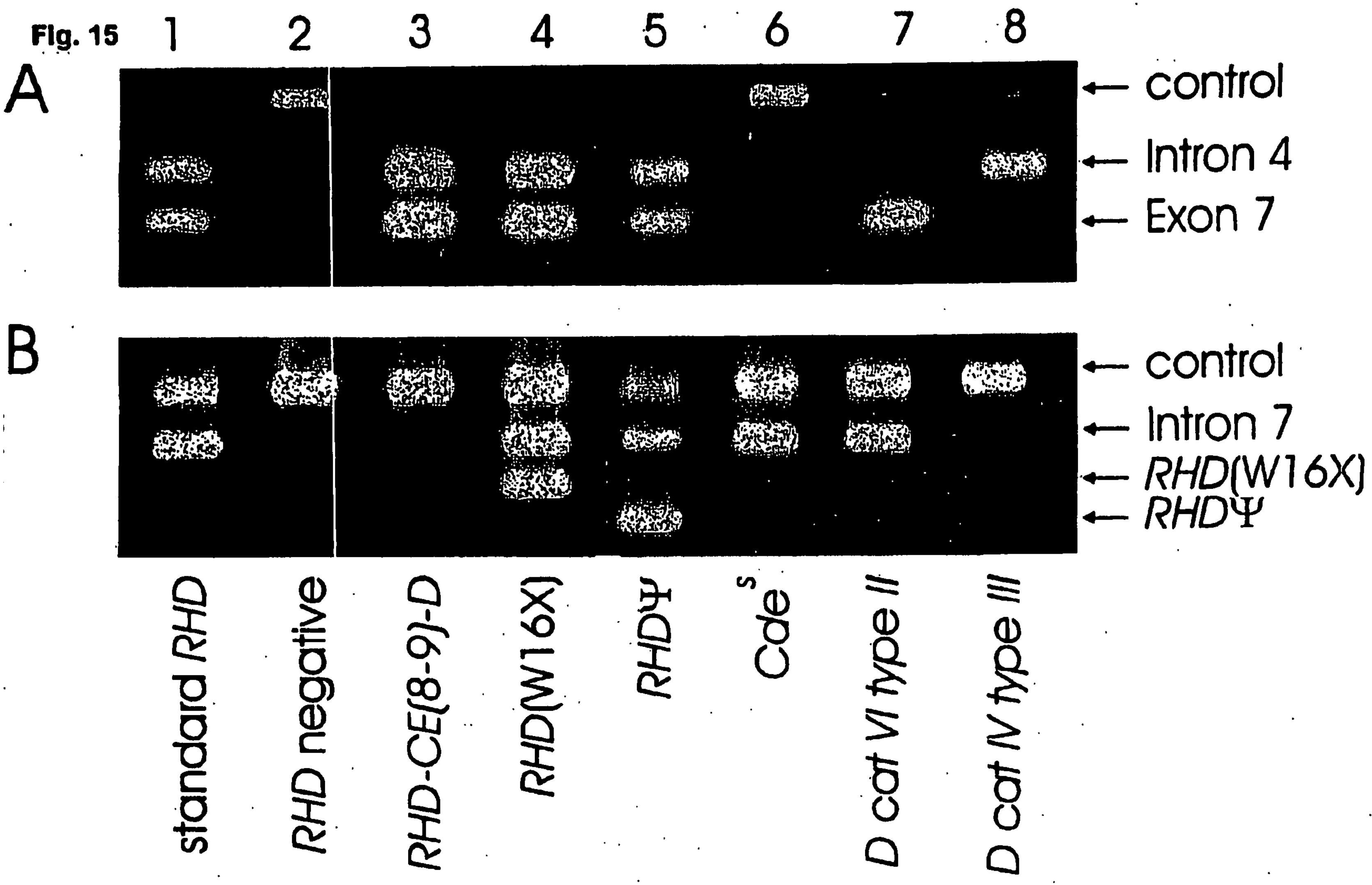


Fig. 15
1
2
3
4
5
6
7
8
A
control
Intron 4
Exon 7
B
control
Intron 7
RHD(W16X)
RHDΨ
standard RHD
RHD negative
RHD-CE(8-9)-D
RHD(W16X)
RHDΨ
Cde^s
D cat VI type II
D cat IV type III

## REFERENCES CITED IN THE DESCRIPTION

*This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.*

### Non-patent literature cited in the description


- **Filbey.** *Acta Obstet Gynecol Scand,* 1995, vol. 74, 687 **[0002]**
- **Bowman, J.** *Semin Perinatol,* 1997, vol. 21, 39 **[0002]**
- **Mourant.** The distribution of the human blood groups and other polymorphisms. Oxford University Press, 1976 **[0002]**
- **Urbaniak.** *Transfusion,* 1981, vol. 21, 64 **[0002]**
- **Cherif-Zahar.** *Hum. Genet.,* 1991, vol. 86, 398 **[0003]**
- **MacGeoch.** *Cytogenet. Cell Genet.,* 1992, vol. 59, 261 **[0003]**
- **Carritt.** *Hum. Mol. Genet.,* 1997, vol. 6, 843 **[0003] [0006]**
- **Flegel.** *Transfus. Med.,* 1998, vol. 8, 281 **[0003] [0005] [0053]**
- **Okuda.** *Biochem. Biophys. Res. Commun.,* 1999, vol. 263, 378 **[0003] [0136]**
- **Gassner.** *Transfusion,* 1997, vol. 37, 1020 **[0004]**
- **Lo.** *Lancet,* 1993, vol. 341, 1147 **[0005]**
- **Cossu.** *Electrophoresis,* 1996, vol. 17, 1911 **[0006] [0053]**
- **Döscher.** *Infusionsther. Transfusionsmed.,* 1999, vol. 26 (1), 31 **[0006]**
- **Huang.** *Am. J. Hum. genet.,* 1996, vol. 58, 133 **[0006]**
- **Fujiwara.** *Hum. genet.,* 1999, vol. 104, 301 **[0006]**
- **Onda.** *Gene,* 1995, vol. 159, 225 **[0006] [0016]**
- **Huang.** *Blood,* 1996, vol. 88, 2326-33 **[0007]**
- **Faas.** *Transfusion,* 1997, vol. 37, 38-44 **[0007] [0152]**
- **Faas.** *Transfusion,* 1996, vol. 36, 506-11 **[0007]**
- **Avent.** *Blood,* 1997, vol. 89, 2568-77 **[0007] [0161]**
- **Andrews.** *Blood,* 1998, vol. 92, 1839-40 **[0007]**
- **Cherif-Zahar.** *Br. J. Haematol.,* 1998, vol. 102, 1263-70 **[0007]**
- **Singleton.** *Blood,* 2000, vol. 95, 12-8 **[0007] [0152]**
- **Singleton.** *Blood,* 2000, vol. 95, 12-18 **[0007]**
- **Okuda.** *J. Clin. Invest.,* 1997, vol. 100, 373-9 **[0007]**
- **Flegel.** *Transfus. Med.,* 1998, vol. 8, 281-302 **[0007]**
- **Hyland.** *Blood,* 1994, vol. 84, 321-4 **[0007]**
- **Blumenfeld.** *Hum. Mutat.,* 1995, vol. 6, 1999 **[0016]**
- **Wagner.** *Blood,* 1998, vol. 91, 2157 **[0016]**
- **Issitt, P.D.** Aplied Blood Group Serology. Scientific Publications, 1985, 239 **[0027]**
- **Blunt, T. ; Daniels, G. ; Carritt, B.** Serotype switching in a partially deleted RHD gene. *Vox Sang,* 1994, vol. 67, 397-401 **[0027]**
- **Faas, B.H.W. ; Becker, E.A.M. ; Wildoer, P. ; Ligthart, P.C. ; Overbeeke, M.A.M. ; Zondervan, H.A. ; Borne, A.E.G.K. ; van der Schoot, C.E.** Molecular background of VS and weak C expression in blacks. *Transfusion,* 1997, vol. 37, 38-44 **[0027]**
- **Daniels, G.L. ; Faas, B.H. ; Green, C.A. ; Smart, E. ; Maaskant-van Wijk, P.A. ; Avent, N.D. ; Zondervan, H.A. ; Borne, A.E. ; van der Schoot, C.E.** The VS and V blood group polymorphisms in Africans: a serologic and molecular analysis. *Transfusion,* 1998, vol. 38, 951-958 **[0027]**
- **Sambrook et al.** Molecular Cloning, A Laboratory Handbook. CSH Press, 1989 **[0057]**
- **Hames ; Higgins.** Nucleic acid hybridization, a practical approach. IRL Press, 1985 **[0057]**
- **Harlow ; Lane.** Antibodies, A Laboratory Manual. CSH Press, 1988 **[0060] [0078]**
- **Osbome et al.** *Curr. Opin. Chem. Biol.,* 1997, vol. I, 5-9 **[0061]**
- **Stall ; Szoka.** *Pharm. Res.,* 1995, vol. 12, 465-483 **[0061]**
- **Winter et al.** *Annu. Rev. Immunol.,* 1994, vol. 12, 433-455 **[0120]**
- **Köln.** *Deutscher Ärzte-Verlag,* 1996 **[0138]**
- **Wagner.** *Infusionsther Transfusionsmed,* 1995, vol. 22, 285-90 **[0138]**
- **Gassner.** *Transfusion,* 1997, vol. 37, 1020-6 **[0149]**
- **Daniels.** *Transfusion,* 1998, vol. 38, 951-8 **[0152]**
- **Wagner, F.F. ; Frohmajer, A. ; Ladewig, B. ; Eicher, N.L ; Lonicer, C.B. ; Müller, T.H. ; Siegel, M.H. ; Flegel, W.A.** Weak D alleles express distinct phenotypes. *Blood,* 2000, vol. 95, 2699-2708 **[0153]**
- **Rouillac, C. ; Colin, Y. ; Hughes-Jones, N.C. ; Beolet, M. ; D'Ambrosio, A.-M. ; Cartron, J.P. ; Le Van Kim, C.** Transcript analysis of D category phenotypes predicts hybrid Rh D-CE-D proteins associated with alteration of D epitopes. *Blood,* 1995, vol. 85, 2937-2944 **[0153]**
- **Flegel.** *Transfusion,* 2000, vol. 40, 428-434 **[0160]**
- **Wagner.** *Infusionsther. Transfusionsmed.,* 1995, vol. 22, 285-90 **[0161]**
- **Andrews KT ; Wolter LC ; Saul A ; Hyland CA.** RhD- trait in a white patient with the RhCCee phenotype attributed to a four-nucleotide deletion in the RHD gene. *Blood,* 1998, vol. 92, 1839 **[0162]**

- **Avent ND ; Martin PG ; Armstrong-Fisher SS ; Liu W ; Finning KM ; Maddocks D ; Urbaniak SJ.** Evidence of genetic diversity underlying Rh D negative, weak D (Du) and partial D phenotypes as determined by multiplex PCR analysis of the RHD gene. *Blood,* 1997, vol. 89, 2568 **[0162]**
- **Avent ND ; Liu W ; Jones JW ; Scott ML ; Voak D ; Pisacka M ; Watt J ; Fletcher A.** Molecular analysis of Rh transcripts and polypeptides from individuals expressing the DVI variant phenotype: an RHD gene deletion event does not generate all DVIccEe phenotypes. *Blood,* 1997, vol. 89, 1779-86 **[0162]**
- **Blumenfeld OO ; Huang CH.** Molecular genetics of the glycophorin gene family, the antigens for MNSs blood groups: multiple gene rearrangements and modulation of splice site usage result in extensive diversification. *Hum Mutat,* 1995, vol. 6, 199 **[0162]**
- **Blunt T ; Daniels G ; Carritt B.** Serotype switching in a partially deleted RHD gene. *Vox Sang,* 1994, vol. 67, 397 **[0162]**
- **Bowman J.** The management of hemolytic disease in the fetus and newborn. *Semin Perinatol,* 1997, vol. 21, 39 **[0162]**
- **Carritt B ; Kemp TJ ; Poulter M.** Evolution of the human RH (rhesus) blood group genes: a 50 year old prediction (partially) fulfilled. *Hum Mol Genet,* 1997, vol. 6, 843 **[0162]**
- **Carritt B ; Steers FJ ; Avent ND.** Prenatal determination of fetal RhD type. *Lancet,* 1994, vol. 344, 205-6 **[0162]**
- **Cherif-Zahar B ; Mattei MG ; Le Van Kim C ; Bailly P ; Cartron JP ; Colin Y.** Localization of the human Rh blood group gene structure to chromosome region 1p34.3-1p36.1 by in situ hybridization. *Hum Genet,* 1991, vol. 86, 398 **[0162]**
- **Cherif-Zahar B ; Bony V ; Steffensen R ; Gane P ; Raynal V ; Goosens D ; Laursen JS ; Varming K ; Jersild C ; Cartron JP.** Shift from Rh-positive to Rh-negative phenotype caused by a somatic mutation within the RHD gene in a patient with chronic myelocytic leukaemia. *Br J Haernatol,* 1998, vol. 102, 1263-70 **[0162]**
- **Colin Y ; Cherif-Zahar B ; Le Van Kim C ; Raynal V ; van Huffel V ; Cartron J-P.** Genetic basis of the RhD-positive and RhD-negative blood group polymorphism as determined by southern analysis. *Blood,* 1991, vol. 78, 2747-52 **[0162]**
- **Cossu G ; Angius A ; Gelfi C ; Righetti PG.** Rh D/d genotyping by quantitative polymerase chain reaction and capillary zone electrophoresis. *Electrophoresis,* 1996, vol. 17, 1911 **[0162]**
- **Daniels GL ; Faas BH ; Green CA ; Smart E ; Maaskant-van Wijk PA ; Avent ND ; Zondervan HA ; Borne AE ; van der Schoot CE.** The VS and V blood group polymorphisms in Africans: a serologic and molecular analysis. *Transfusion,* 1998, vol. 38, 951-8 **[0162]**
- **Döscher A ; Schunter F ; Müller TH.** Quantitativae PCR to assess RHD zygosity. *Infusionsther Transfusionsmed,* 1999, vol. 26 (1), 31 **[0162]**
- **Faas BHW ; Becker EAM ; Wildoer P ; Ligthart PC ; Overbeeke MAM ; Zondervan HA ; Borne AEGK ; van der Schoot CE.** Molecular background of VS and weak C expression in blacks. *Transfusion,* 1997, vol. 37, 38 **[0162]**
- **Faas BHW ; Beckers EAM ; Simsek S ; Overbeeke MAM ; Pepper R ; van Rhenen DJ ; Borne AEGK ; van der Schoot CE.** Involvement of Ser103 of the Rh polypeptides in G epitope formation. *Transfusion,* 1996, vol. 36, 506 **[0162]**
- **Filbey D ; Hanson U ; Wesstrom G.** The prevalence of red cell antibodies in pregnancy correlated to the outcome of the newborn: a 12 year study in central Sweden. *Acta Obstet Gynecol Scand,* 1995, vol. 74, 687 **[0162]**
- **Flegel WA ; Khull SR ; Wagner FF.** Primary anti-D immunization by weak D type 2 RBCs. *Transfusion,* 2000, vol. 40, 428-434 **[0162]**
- **Flegel WA ; Wagner FF ; Müller TH ; Gassner C.** Rh phenotype prediction by DNA typing and its application to practice. *Transfus Med,* vol. 8, 281 **[0162]**
- **Fujiwara H ; Okuda H ; Omi T ; Iwarnoto S ; Tanaka Y ; Takahashi J ; Tani Y ; Minakami H ; Araki S ; Sato I.** The STR polymorphisms in intron 8 may provide information about the molecular evolution of RH haplotypes. *Hum Genet,* 1999, vol. 104, 301 **[0162]**
- **Gassner C ; Schmarda A ; Kilga-Nogler S ; Jenny-Feldkircher B ; Rainer E ; Müller TH ; Wagner FF ; Flegel WA ; Schonitzer D.** RhesusD/CE typing by polymerase chain reaction using sequence-specific primers. *Transfusion,* 1997, vol. 37, 1020 **[0162]**
- **Huang CH.** Human DVI category erythrocytes: correlation of the phenotype with a novel hybrid RhD-CE-D gene but not an internally deleted RhD gene. *Blood,* 1997, vol. 89, 1834-9 **[0162]**
- **Huang CH ; Reid ME ; Chen Y ; Coghlan G ; Okubo Y.** Molecular definition of red cell Rh haplotypes by tightly linked SphI RFLPs. *Am J Hum Genet,* 1996, vol. 58, 133 **[0162]**
- **Huang CH.** Alteration of RH gene structure and expression in human dCCee and DCW- red blood cells: phenotypic homozygosity versus genotypic heterozygosity. *Blood,* 1996, vol. 88, 2326 **[0162]**
- **Hyland CA ; Wolter LC ; Saul A.** Three unrelated Rh D gene polymorphisms identified among blood donors with Rhesus CCee (r'r') phenotypes. *Blood,* 1994, vol. 84, 321-4 **[0162]**
- **Kemp TJ ; Poulter M ; Carritt B.** Microsatellite variation within the human RHCE gene. *Vox Sang,* 1999, vol. 77, 159 **[0162]**

- **Lo Y-MD ; Bowell PJ ; Selinger M ; Mackenzie IZ ; Chamberlain P ; Gillmer MDG ; Littlewood TJ ; Fleming KA ; Wainscoat JS.** Prenatal determination of fetal RhD status by analysis of peripheral blood of rhesus negative mothers. *Lancet,* 1993, vol. 341, 1147 **[0162]**
- **Maaskant-van Wijk PA ; Hemker MB ; Douglas-Berger L et al.** Ethnic variability of the Rhesus system. *Transfusion,* 1999, vol. 39 (10S), 103S-4S **[0162]**
- **Maaskant-van Wijk PA ; Faas BHW ; de Ruijter JAM ; Overbeeke MAM ; Borne AEGK ; van Rhenen DJ ; van der Schoot CE.** Genotyping of RHD by multiplex polymerase chain reaction analysis of six RHD-specific exons. *Transfusion,* 1998, vol. 38, 1015-21 **[0162]**
- **Maaskant-van Wijk PA ; Beckers EAM ; van Rhenen DJ ; Mouro I ; Colin Y ; Carton J-P ; Faas BHW ; van der Schoot CE ; Apoil PA ; Blancher A et al.** Evidence that the RHD(VI) deletion genotype does not exist. *Blood,* 1997, vol. 90, 1709-11 **[0162]**
- **MacGeoch C ; Mitchell CJ ; Carritt B ; Avent ND ; Ridgwell K ; Tanner MJ ; Spurr NK.** Assignment of the chromosomal locus of the human 30-kDal Rh (rhesus) blood group-antigen-related protein (Rh30A) to chromosome region 1p36,13-p34. *Cytogenet Cell Genet,* 1992, vol. 59, 261 **[0162]**
- **Mourant AE ; Kopec AC ; Domaniewska-Sobczak K.** The distribution of the human blood groups and other polymorphisms. Oxford University Press, 1976 **[0162]**
- **Okuda H ; Suganuma H ; Tsudo N ; Omi T ; Iwamoto S ; Kajii E.** Sequence analysis of the spacer region between the RHD and RHCE genes. *Biochem Biophys Res Commun,* 1999, vol. 263, 378 **[0162]**
- **Okuda H ; Kawano M ; Iwamoto S ; Tanaka M ; Seno T ; Okubo Y ; Kajii E.** The RHD gene is highly detectable in RhD-negative Japanese donors. *J Clin Invest,* 1997, vol. 100, 373 **[0162]**
- **Onda M ; Fukuda M.** Detailed physical mapping of the genes encoding glycophorins A, B and E, as revealed by P1 plasmids containing human genomic DNA. *Gene,* 1995, vol. 159, 225 **[0162]**
- **Reboul J ; Gardiner K ; Monneron D ; Uze G ; Lutfalla G.** Comparative genomic analysis of the interferon/interleukin-10 receptor gene cluster. *Genome Res,* 1999, vol. 9, 242 **[0162]**
- **Salvignol I ; Calvas P ; Socha WW ; Colin Y ; Le Van Kim C ; Bailly P ; Ruffie J ; Cartron JP ; Blancher A.** Structural analysis of the RH-like blood group gene products in nonhuman primates. *Immunogenetics,* 1995, vol. 41, 271 **[0162]**
- **Singleton BK ; Green CA ; Avent ND ; Martin PG ; Smart E ; Daka A ; Narter-Olaga EG ; Hawthorne LM ; Daniels G.** The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in africans with the Rh D- negative blood group phenotype. *Blood,* 2000, vol. 95, 12 **[0162]**
- **Urbaniak SJ ; Robertson AE.** A successful program of immunizing Rh-negative male volunteers for anti-D production using frozen/thawed blood. *Transfusion,* 1981, vol. 21, 64 **[0162]**
- **Wagner FF ; Gassner C ; Müller TH ; Schönitzer D ; Schunter F ; Flegel WA.** Three molecular structures cause Rhesus D category VI phenotypes with distinct immunohematologic features. *Blood,* 1998, vol. 91, 2157 **[0162]**
- **Wagner FF ; Kasulke D ; Kerowgan M ; Flegel WA.** Frequencies of the blood groups ABO, Rhesus, D category VI. Kell, and of clinically relevant high-frequency antigens in South-Westem Germany. *Infusionsther Transfusionsmed,* vol. 22, 285 **[0162]**
- **Wagner FF ; Gassner C ; Muller TH ; Schonitzer D ; Schunter F ; Flegel WA.** Molecular basis of weak D phenotypes. *Blood,* 1999, vol. 93, 385 **[0162]**
- **Wagner FF ; Frohmajer A ; Ladewig B ; Eicher Nt ; Lonicer CB ; Müller TH ; Siegel MH ; Flegel WA.** Weak D alleles express distinct phenotypes. *Blood,* 2000, vol. 95, 2699-2708 **[0162]**
- **Wagner FF ; Flegel WA.** RHD gene deletion occurred in the Rhesus box. *Blood,* 2000 **[0162]**
- Wissenschaftlicher Beirat der Bundesärztekammer. Richtlinien zur Blutgruppenbestimmung und Bluttransfusion. Köln: Deutscher Ärzte-Verlag, 1996 **[0162]**
- **Legler TJ ; Kohler M ; Mayr WR ; Panzer S ; Ohto H ; Fischer GF.** Genotyping of the human platelet antigen systems 1 through 5 by multiplex polymerase chain reaction and ligation-based typing. *Transfusion,* May 1996, vol. 36 (5), 426-31 **[0162]**

patsnap

| | | | |
|---|---|---|---|
| 专利名称(译) | RHD阴性基因座的分子结构 | | |
| 公开(公告)号 | EP1780217B1 | 公开(公告)日 | 2010-03-31 |
| 申请号 | EP2006026286 | 申请日 | 2000-10-31 |
| [标]申请(专利权)人(译) | DRK BLUTSPENDEDIENST巴符州HESSEN GEMEINNUTZIGE | | |
| 申请(专利权)人(译) | DRK-BLUTSPENDEDIENST巴登 - 符腾堡州，HESSENGEMEINNÜTZIGEGMBH | | |
| 当前申请(专利权)人(译) | DRK-BLUTSPENDEDIENST巴登 - 符腾堡州，HESSENGEMEINNÜTZIGEGMBH | | |
| [标]发明人 | FLEGEL WILLY A<br>WAGNER FRANZ F | | |
| 发明人 | FLEGEL, WILLY A.<br>WAGNER, FRANZ F. | | |
| IPC分类号 | C07K14/47 C12N15/12 C12Q1/68 G01N33/53 A61K39/395 A61P15/00 C07K14/705 C07K16/18 C12N5/10 C12N7/00 C12N15/09 C12P21/02 C12P21/08 C12Q1/02 G01N33/566 | | |
| CPC分类号 | A61P15/00 C07K14/705 C12Q1/6883 C12Q2600/156 C12Q2600/16 C12Q2600/172 C12N15/52 | | |
| 代理机构(译) | 法思博事务所 | | |
| 优先权 | 1999121686 1999-11-02 EP<br>2000111696 2000-05-31 EP | | |
| 其他公开文献 | EP1780217A1 | | |
| 外部链接 | Espacenet | | |


摘要(译)

本发明涉及代表Rhesus基因座的核酸分子结构，其包含RHD，SMP1和RHCE基因和/或恒河猴盒，优选混合恒河猴盒，上游恒河猴盒和/或下游恒河猴盒。 。此外，本发明涉及特异性检测常见RHD阴性单倍型的方法。本发明还涉及D阴性个体中RHD阳性降低型的检测。已经鉴定了RHD基因中的各种突变，其允许开发诊断工具。本发明还涉及寡核苷酸，其特异性杂交杂交盒，优选断裂点或断裂点区域或上游和下游恒河猴盒。另外，本发明涉及包含或使用上述本发明化合物的试剂盒。


**Fig. 10**

**Upstream Rhesus box of D-positives**

```
5' ctagaaaacactttgtcattttagaggtgtta
(start of Rhesus box)
tccaatgttcgcgcaggcactggagtcagagaaaatggagttgaatcctttctctgccactc
tttgaggagaatctcaccatttattatgcactgtagaatacaacaataaaatacagccatgt
accacataacaacatcttggtaaacaacagactgcatatatgatggtggtcatccagtaagc
taaggttaatttattattattccctttttttttttcttttttttgagatgtagtcttactctg
tcacccaggctagagtgcaatggcaccatcttggctcactgcaacctctgcctcctgggttc
aagcgaatctcctgcctcagcctccgaagtagctgggaattacaggcacccaccacatctgg
ctaatttttgtatttttagtaaagatggggtttcaccatgttggccaggctgatctcaaac
tcctgacctcaagtgatctgcctgcctcggcctcccaaagtgctgggaccataggcctgagc
cactgtgcccggccttgtttgcttttttaacagttaacagtgtgctcatagaaactgctttg
acatgactgcaatcatgtgcttcatagaaacttaattagattataccactagagtcttcaga
ttttatacttttttttttgaaacggagtctcactctgtcaccaggctggagtgcagtgccg
caatctcagctcgccgcaacctccgcctcccaggttcaagtgattctcctgcctcagcctcc
cgagtagctgggattacaagtgcacactaccacgcccagctaattttgcatttttactaga
cagggtttcaccatgttggctaggatagtttcaccaggatctcttggcctcatgatcagcct
gcctcggcctcccaaagtgctgggattacaggtgtgagccaccgtgcccagcctatacttcc
ctttttgaataccatttggcgttttgaagaattaacagctttgtgaacgtggcagtgcttgt
gattcaggcttccactgagaccaaggggagaacctggttgcaggacaaacagacggacagcg
tgtggcagtgtttaaatgctcttctgaaggctgatacgacagctctctgtgcactgattgca
tacgcatcccaagattatattattgttttctattgctatgtgtcacactttgccaaacagga
tgtggaaaatgaataagcggttttcttaggcacttcttaacagacaattggtcaaaatgaac
tccattgcttaagaaacacataaacaccatttagtcactgaatatagctatatgtatggttg
ctactatggggaatcttgttttgccaattttctttgaaaattctggcagaccaaggttcttt
ttgtttacacaatacttgaaaaataaaaatgaacaagccaacaaactaccaagttttcactt
acataaatgtagttacatacagaaaatgtgactgtgaattttttctaggacttttaaactat
aagcactatttgcacgaaagagaaccaatctatcaattacaaactcacataattttacagat
ttttttttccctacacagcacataaaacagaaggaatttgaagccaccctccaaacacaggg
gaaggaggctgtgtgtatatcctcattgtctttcacattctaaggtggttccactcagtgac
tgaaatccttaagtgttgtattagtcggcttgggctaccataacagcagcttaaactgttgt
taagccactcagacttaaacaacagaaatttatttccttatagttctggaggctggaagttc
aaggtgccggcaaggctggtttctggtgagacctctctccctgtcttgcagatggctgcctc
ctccctgtgtcctcatagagcctgtcctctgcttttacacttctggtgtcatcttcctttttt
tttttttttttgagacagagtctcgctctatcgcccaggctggagtgcagtggcccgatcga
tctcggctcactgcaacctctgcctcccaggttcaagcaattctcctgcctcagcctcccga
gtagctgggactacaggtgcccgccatcatgtctggctaattttgtatttttagtagagac
agggtttcaccatattggccaggctggtctccaactcctgaccttgtcatctgcctgcctcg
gcctcccaaagtgctaggattacaggcgtgagccaccgcacccggcctctttctcttcttat
aaggacaccagtcctattagattagggctccaccctcatgacctcatttgaccttaactatt
atttctttaaagcacctatttccaaatatagtcactttaggggttagggcttcaaaatatga
atctgagggagatcaattcagtaaatagcagtagtcattaacggacaatatatacaaagata
atttcgtgattactgtccttatgcataaatgtcctcagtgttccactgcctttatccagatt
tactatcacaaagactttgctctgagaaaaatgtgatttctttcttttttttttttttttga
gacagagtctcactctgtcacccaggctggagtgcagtggtgcaatctcggctcactgcaat
ctccgcctcccaggttcacgccattctcttgcctcagtctcccgagtagctgggcctacagg
```