Supplementary Information

Patients with Asian-type DEL can safely be transfused using RhD-positive blood

SUPPLEMENTARY MATERIALS

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SUPPLEMENTARY METHODS

1. Routine serological typing for Asian-type DEL

In this study, RhD antigen was first typed using two anti-D reagents (Clone Rum-1, IgM, Shanghai Hemo-Pharmaceutical & Biological Co., LTD, China; Clone TH-28/MS-26, IgM/IgG, Millipore, UK). For samples with a serologically apparent D– phenotype after excluding weak D and partial D, DEL phenotyping was diagnosed by adsorption/elution testing as described previously[1, 2] using two anti-D mAbs (HM16, IgG, Diagast, France; MS26, IgG, Shanghai Hemo-Pharmaceutical & Biological, China). For each assay, blood samples from Asian-type DEL and true D– donors were used as positive and negative controls, respectively.

2. Establishment of a high-resolution melting (HRM) method for routine detection of Asian-type DEL allele

For the laboratory test for Asian-type DEL, the detection of the *RHD*1227A* allele is necessary because the usual serological adsorption/elution test is a time-consuming manual operation and cannot distinguish Asian-type DEL from other types of DELs with different genetic backgrounds. Moreover, traditional genotyping methods (such as PCR-SSP[3]) are time-consuming and not convenient for routine testing of the Asian-type DEL allele. Therefore, we developed a high-resolution melting (HRM) method to detect the region of exon 9 of the *RHD* gene covering the specific synonymous mutation (c.1227G>A, p. Lys409Lys) responsible for the most common Asian-type DEL phenotype. The region of exon 9 of the *RHD* gene harboring the mutation was amplified. A pair of *RHD*-specific primers (*RHD*-F: ATATGGAAAGCACCTCATGA; *RHD*-R: AAACAGCAAGTCAACATATATACT encompassing 139 bp; NC_000001.11) was designed and synthesized. A 20-μL PCR mixture consisted of 30 ng of genomic DNA, 3 mM $MgCl_2$, 10 μL 2×LightCycler® 480 High Resolution melting master mixture (Roche Diagnostics GmbH, Mannheim, Germany), 0.3 μM forward and reverse primers and 6 ng of spiking DNA from D+ controls with the normal *DD* genotype. PCR was carried out for 10 sec at 95°C, followed by 45 cycles of denaturation at 95°C for 10 sec, annealing at 60°C for 15 sec, and extension at 72°C for 10 sec; an HRM step at 95°C for 1 min and temperature reduction to 40°C for 1 min was performed, followed by continuous acquisition at 25 times per degree. The HRM experiment was continuously performed in a closed tube using a LightCycler® 480 II (Roche Diagnostics International Ltd, Rotkreuz, Switzerland), and data analysis was conducted using LightCycler 480 software v1.5.1.62 (Roche Diagnostics International Ltd).

The well-defined reference Asian-type DEL samples with the Asian-type DEL phenotype and different known genotypes carrying the *RHD*01EL.01* (*RHD*1227A*) allele (homozygous *RHD*1227A/1227A* genotype (n = 3), heterozygous *RHD*1227A/1227G* genotype (n = 1), *RHD*1227A/01N.01* genotype (n = 10)) and wild-type samples with *RHD*1227G/1227G* genotype were used to develop and validate the HRM method. The Asian-type DEL phenotype of reference samples was detected by the serological adsorption/elution method. The *RHD* genotypes of reference samples were determined by using the proven *RH*-multiplex ligation-dependent probe amplification (*RH*-MLPA) assay and sequencing of *RHD* exon 9 as described previously[4, 5]. In each test, the DNA samples of Asian-type DEL individuals with three different genotypes and individuals with the wild-type *RHD* genotype were tested in parallel with unknown samples for HRM genotyping as controls. Two hundred *RHD*1227A* allele carriers detected by HRM were sequenced for *RHD* exon 9 by Sanger sequencing to validate the HRM method.

As a result, the reference samples were classified into four groups with different melting curves (**Fig. S1**). When the HRM melting curves of tested samples were classified into the melting curves of Asian-type DEL reference samples with three different genotypes, the *RHD*1227A* allele was detected. If a tested sample was shown to have a different melting curve compared with Asian-type DEL reference samples, the exon 9 of the *RHD* gene was amplified and sequenced to clarify the variants except for the c.1227A. The 200 *RHD*1227A* allele carriers identified by HRM were confirmed to have the c.1227A variant by Sanger sequencing.

In summary, HRM is a simple, rapid, inexpensive, and reliable method for routine testing of the Asian-type DEL allele. Thirty samples with triplicate can be run in a 96-well plate within two and a half hours, including less than one hour of genomic DNA extraction and pipetting steps.

3. Inclusion and exclusion criteria for participants of clinical trial

Inclusion criteria for eligible participants were as follows: (1) Asian-type DEL blood group; (2) male patients, or female patients ⩾ 49 years of age, or female with severe illness and no plan for further pregnancy; (3) needing blood transfusion in line with guidelines for internal medicine or surgery; and (4) signed voluntary informed consent for blood transfusion treatment before the trial.

The exclusion criteria were: (1) adverse reaction in a previous transfusion; (2) allergies to blood products or immunodeficiency diseases; (3) needing massive blood transfusion for acute blood loss; (4) positive pregnancy test results; (5) conscious dysfunction or severe mental illness; and (6) unsuitability for the study, as estimated by the principal investigators.

The main recruitment strategy was to inform the participants the benefit of the trial. D+ blood supply for Asian-type DEL patients, can be guaranteed, as opposed to rare D– blood, which is often in short supply in China, and many Asian countries.

4. PCR amplification and Sanger sequencing analysis of *RHD* transcripts covering c.1227A variant and exon 9

The region of *RHD* exon 9 to the 3'-UTR was amplified using primers (forward: 5'-CTGACAGGTTTGCTCCTAAATCTT-3'; reverse: 5'- CTCTGACTCCAGTGCCTGCGCG-3'[6]) with GoTaq Colorless Master Mix (Promega, Madison, USA). The PCR conditions were: 5 minutes at 94°C; 40 cycles of 30 seconds at 94°C, 30 seconds at 58°C, 30 seconds at 72°C; final extension at 72°C for 5 minutes. The PCR product was sequenced by the Sanger method, and the sequences were analyzed with the SeqManII program of the Lasergene package (DNASTAR Inc., Madison, WI, USA).

5. Nanopore sequencing analysis of full-length *RHD* transcripts

The whole coding region of *RHD* cDNA was amplified using primers[6] (Forward_*RHD*_ 5'-UTR: 5'-CTGCACAGAGACGGACACAG-3'; Reverse_3'-UTR: 5'-CTCTGACTCCAGTGCCTGCGCG -3') (Fig. 3A) and LongAmp Taq 2X Master Mix (New England Biolabs). The PCR conditions were: 30 seconds at 95°C; 15-25 cycles of 15 seconds at 95°C, 30 seconds at 57°C, 1 minute and 20 seconds at 65°C; final extension at 65°C for 5 minutes. The PCR products were then subjected to library preparation for Nanopore sequencing (Oxford Nanopore Technologies, Oxford, England). Briefly, all PCR products were cleaned with AMPure XP beads (Beckman Coulter Inc., Brea, CA, USA). DNA end-repair was performed using NEBNext Ultra II End Repair/dA-Tailing Module (New England Biolabs), followed by purification with AMPure XP beads and ligation of barcodes with Native Barcoding Expansion 1-12 (EXP-NBD104, Oxford Nanopore Technologies, Oxford, England) and Blunt/TA Ligase Master Mix (New England Biolabs). DNA sequencing Adapter Mix (Oxford Nanopore Technologies, Oxford, England) was ligated to the libraries with NEBNext Quick Ligation Module (New England Biolabs). The libraries were incubated with AMPure XP beads and washed twice with Short Fragment Buffer (EXP-SFB001, Oxford Nanopore Technologies, Oxford, England). Finally, the libraries were sequenced with the R10.3 Oxford nanopore flow cell using the SQK-LSK109 method and a MinION or MinION Mk1C sequencer (Oxford Nanopore Technologies, Oxford, England). Base calling of raw Nanopore sequencing data was performed using Guppy v 4.4.2 (--barcode_kits "EXP-NBD114" -c dna_r10.3_450 bps_hac.cfg --chunk_size 4000 --chunks_per_runner 1000 --device). The reads were mapped to the human genome (GRCh37.75) by minimap2 v2.18[7]. The aligned read bam files were indexed and visually inspected using Integrative Genomics Viewer. *RHD* transcripts were identified, quantified, and analyzed using FLAIR v1.5 (Full-Length Alternative Isoform analysis of RNA)[8].

6. Expression of major *RHD* variant transcripts *in vitro*

Wildtype (wt) pHeftig-*RHD* (*RHD*1227G*) and pSINK-*RHAG*, carrying the complete coding sequences of wt *RHD* (NM_016124.6) and wt *RHAG* (NM_000324.3), respectively, were kindly provided by Prof. C. Ellen van der Schoot (University of Amsterdam, the Netherlands). The seven most common *RHD* spliced transcripts, including exon 9 deletion, exon 8/9 deletion, exon 7/9 deletion, exon 7/8/9 deletion, exon 9 deletion with insertion of 170 bp of intron 7, exon 8/9 deletion with insertion of 170 bp of intron 7, and the full-length *RHD* transcript carrying the c.1227A variant, were synthesized and cloned into the pHeftig vector by Sangon Biotech

(Shanghai, China). HEK 293T cells, which do not express endogenous RhAG and RhD, were transiently transfected with *RHD* constructs individually with the wt *RHAG* construct. Forty-eight hours after transfection, surface expression of RhD antigens was analyzed using a panel of seven anti-D mAbs for the systematic detection of D epitopes (Clone P3×249, P3×290, and HM16, Diagast, France; MS26, Shanghai Hemo-Pharmaceutical & Biological, China; LHM169/81, LHM76/58, and LHM76/59, kindly provided by Prof. C. Ellen van der Schoot, University of Amsterdam, the Netherlands) by flow cytometry. An Alexa Fluor 647 goat anti-human IgG (H + L) antibody (1:50 dilution, Life Tech, Eugene, OR) was used as the secondary antibody. Data acquisition and analysis were performed with a BD FACSCanto II flow cytometer and FlowJo v.7.6 software, respectively.

SUPPLEMENTARY FIGURES

Figure S1

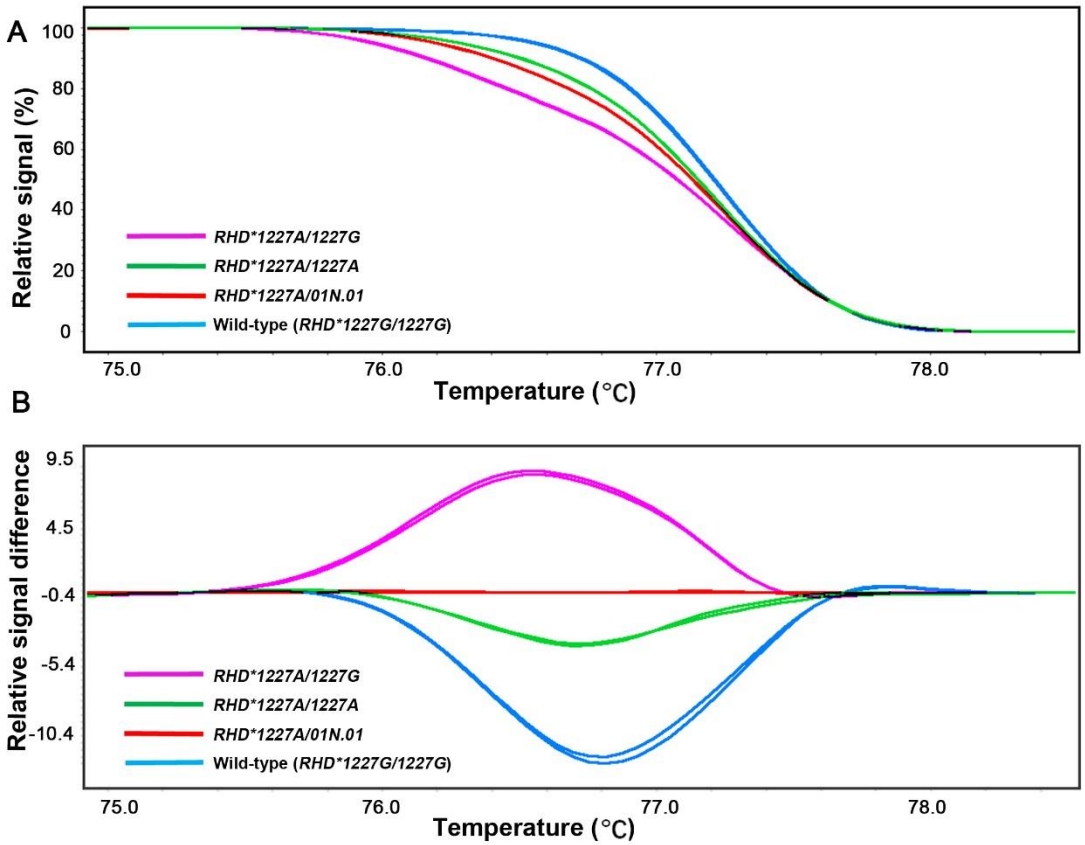

Figure S1. High-resolution melting curve analysis for Asian-type DEL samples

Three Asian-type DEL samples carrying the *RHD*01EL.01* (*RHD*1227A*) allele (homozygous *RHD*1227A/1227A*, heterozygous *RHD*1227A/1227G* and *RHD*1227A/RHD*01N.01*) and a wild-type (*RHD*1227G/1227G*) sample were used. The differential melting properties of representative samples are shown using (A) normalized and temperature-shifted melting curves and (B) normalized and temperature-shifted difference plots. For each sample, two technical replicates were analyzed.

Figure S2

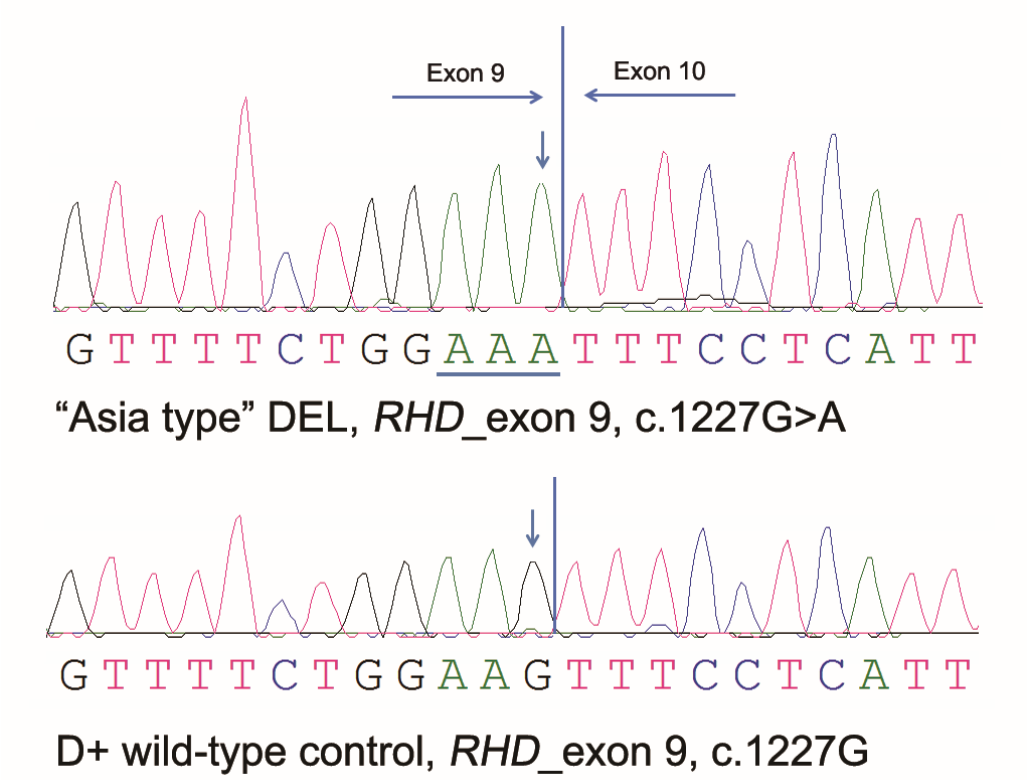

Figure S2. Sanger sequencing of *RHD* transcript fragment carrying exon 9 and the c.1227A variant in Asian-type DEL individuals

The mRNA was extracted from the cultured erythroblast of Asian-type DEL individuals and D+ controls. cDNA fragments spanning exon 9 to 3'UTR were amplified and sequenced.

Figure S3

> Homo sapiens Rh blood group D antigen (RHD), transcript covering the whole coding region and a c.1227A variant

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Figure S3. The raw sequence of full-length *RHD* transcript obtained in Asian-type DEL individuals by Nanopore sequencing

Figure S4

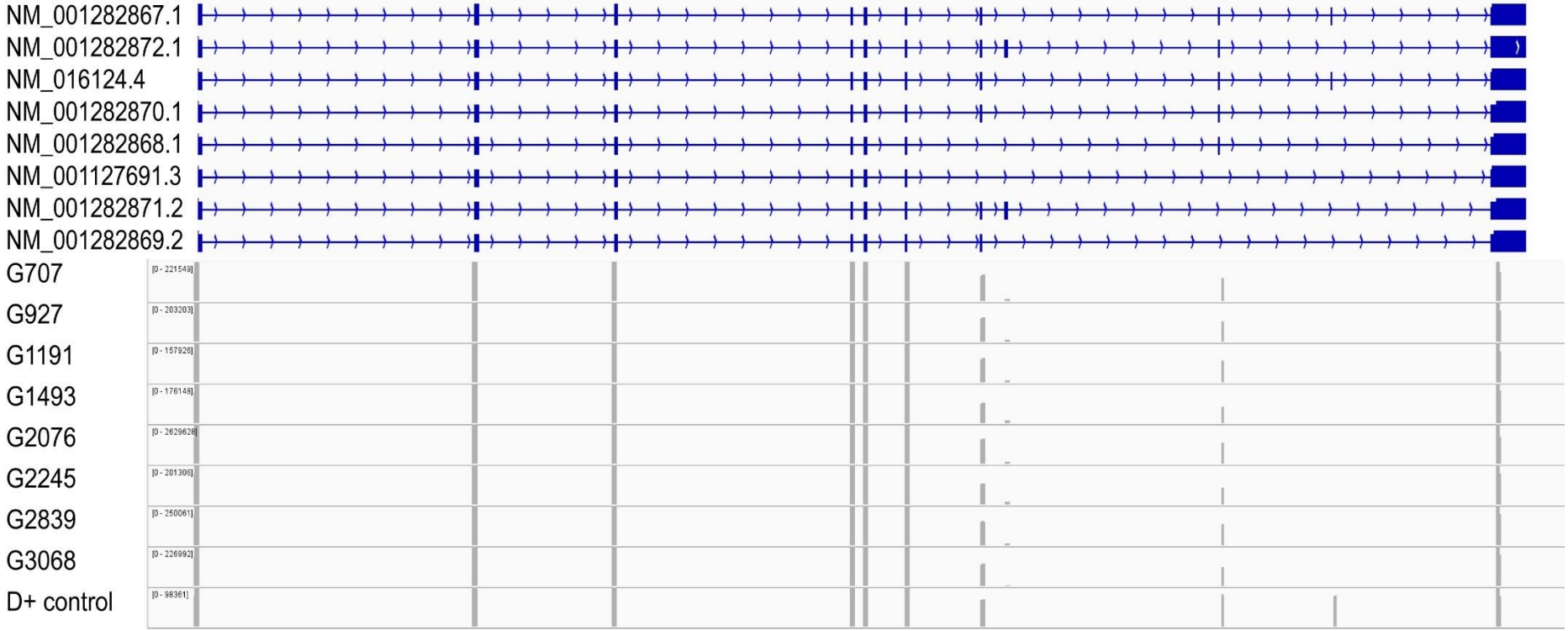


Fig S4. Alignment to the different reference *RHD* transcripts in Nanopore sequencing

Figure S5

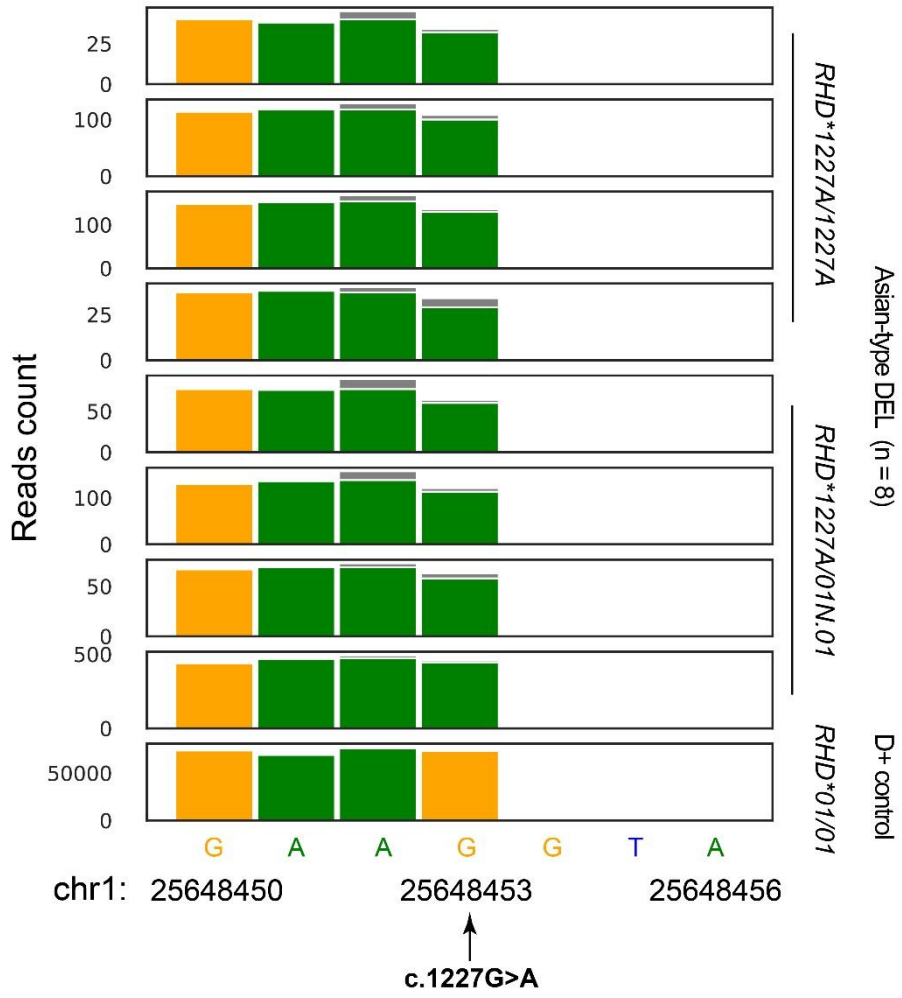


Figure S5. The A/G ratio of *RHD* c.1227G>A variant of full-length transcript detected by Nanopore sequencing

G nucleotide is shown in yellow, and A nucleotide is shown in green. Other nucleotides except for G or A are shown in grey.

Figure S6

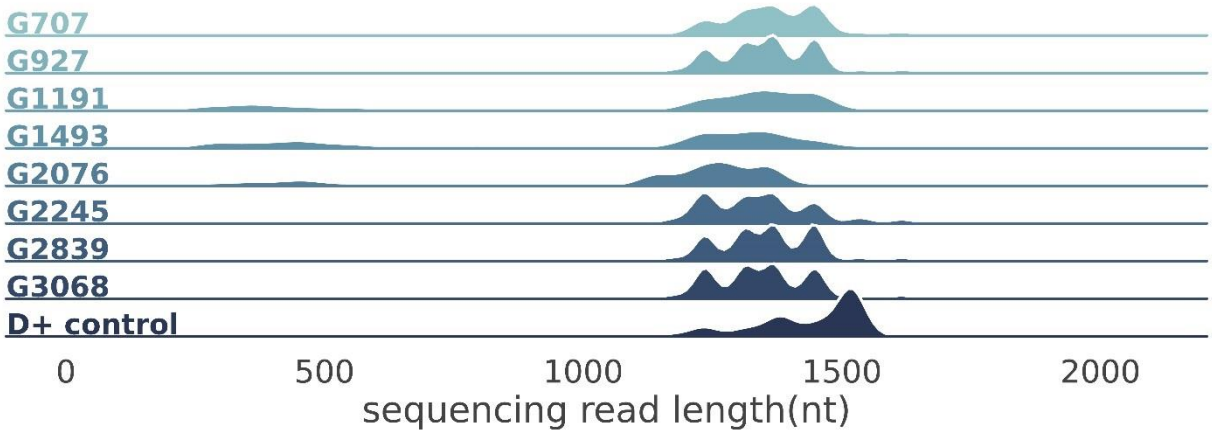


Fig S6. Sequencing read length of Nanopore sequencing

Figure S7

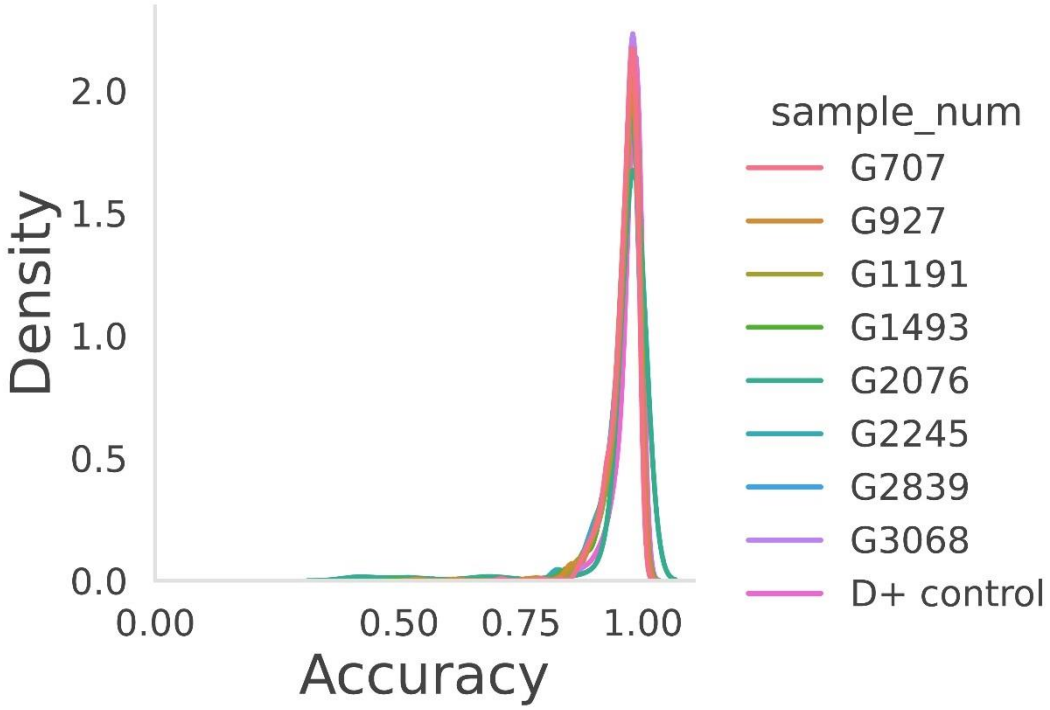
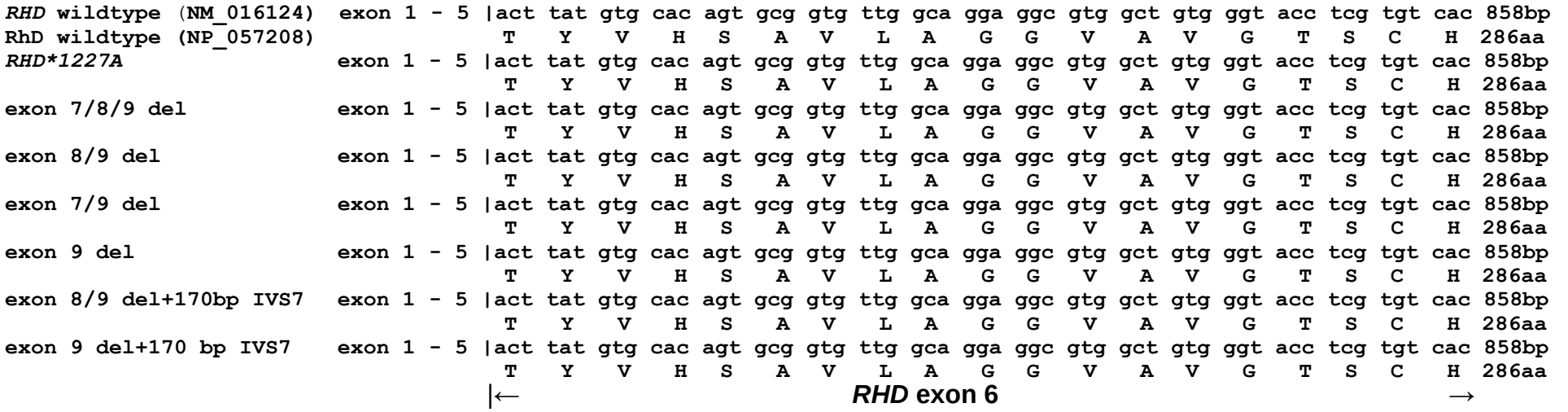


Fig S7. sequencing accuracy rate of Nanopore sequencing

With continuous technology development, the error rate of Nanopore sequencing raw reads is at 5% achieving 95% accuracy.

Figure S8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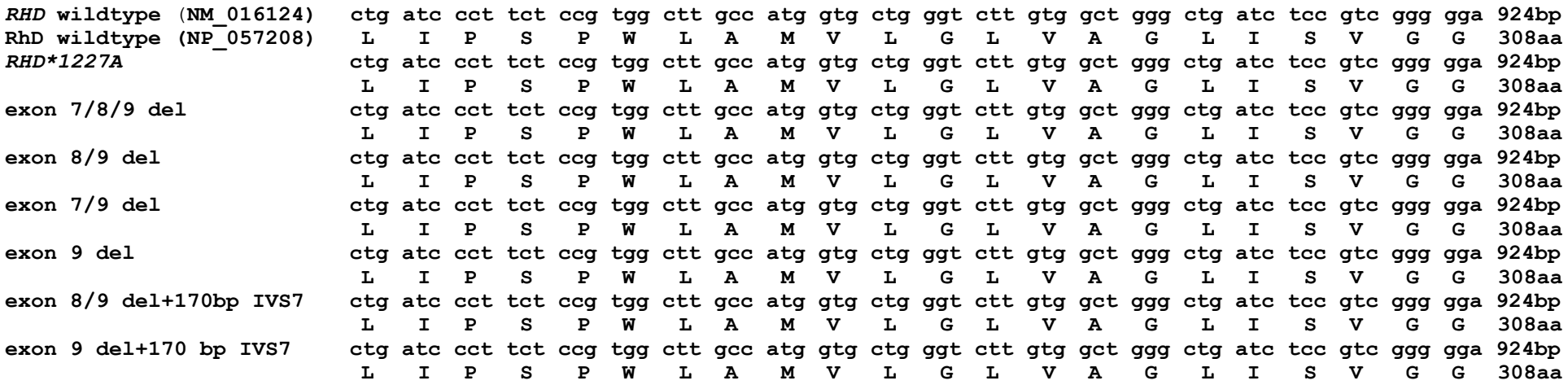
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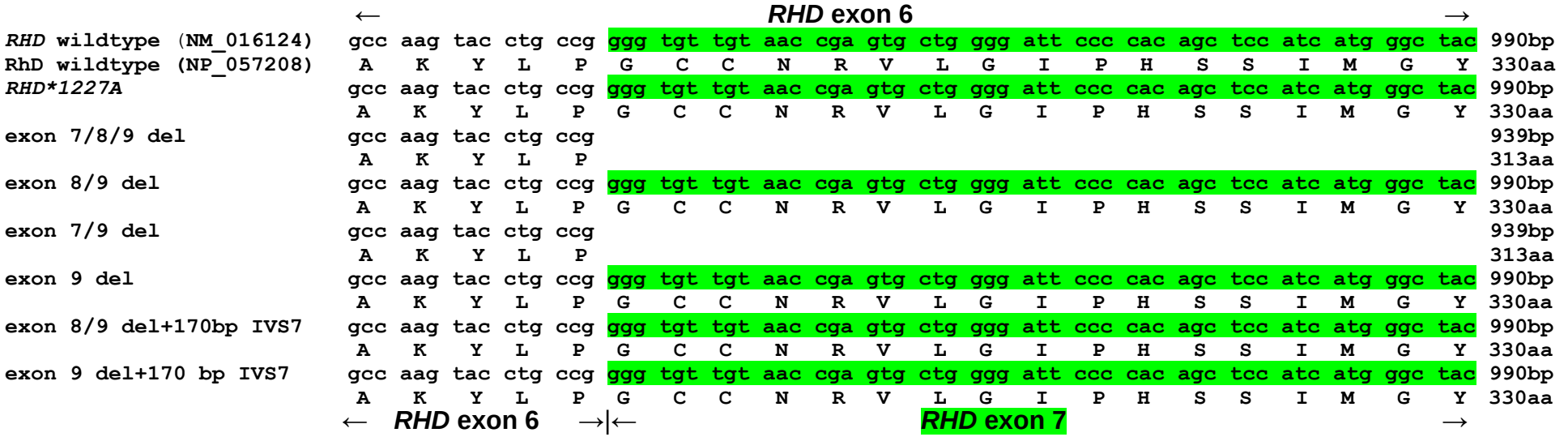
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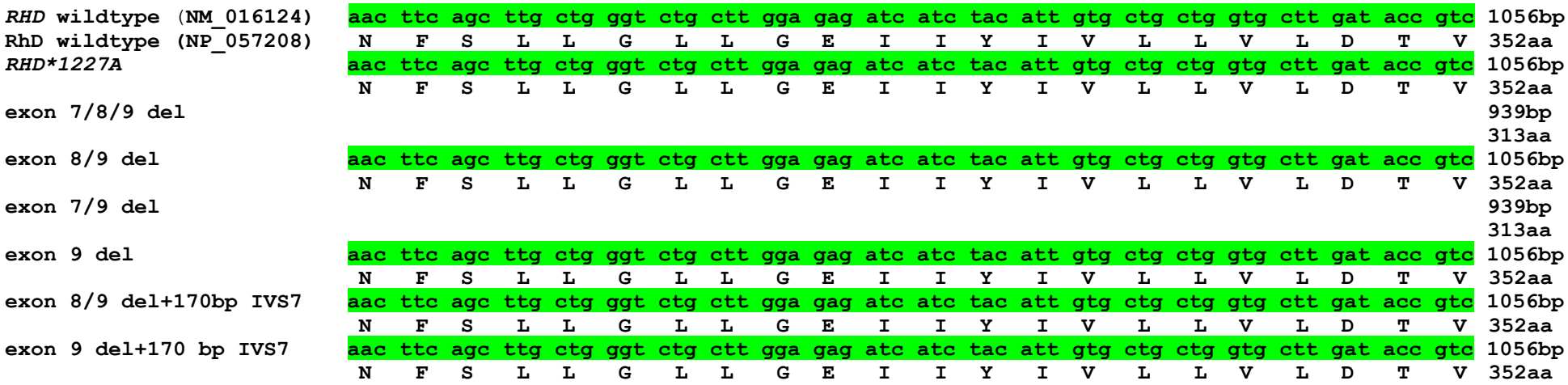
RHD exon 7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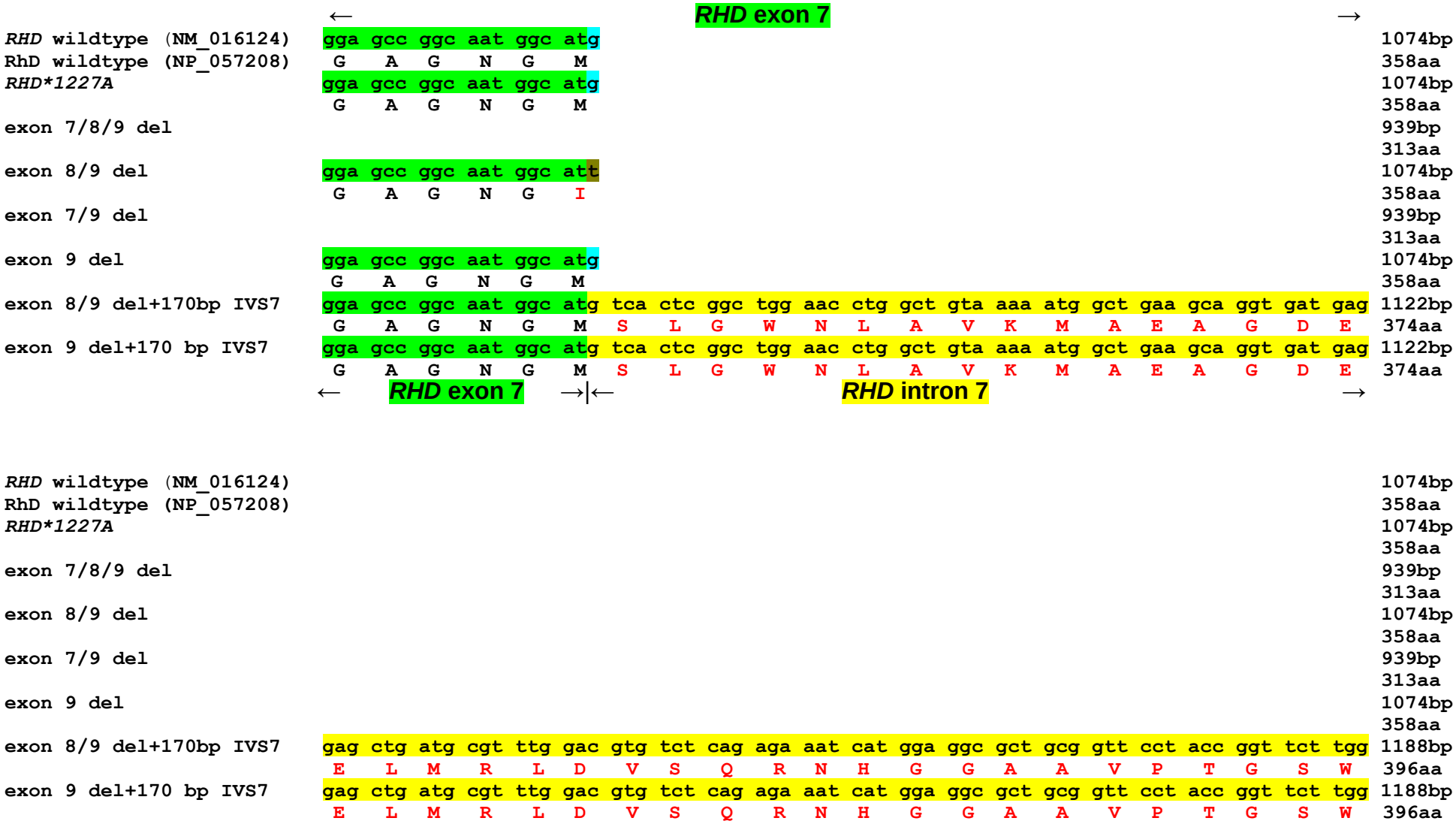
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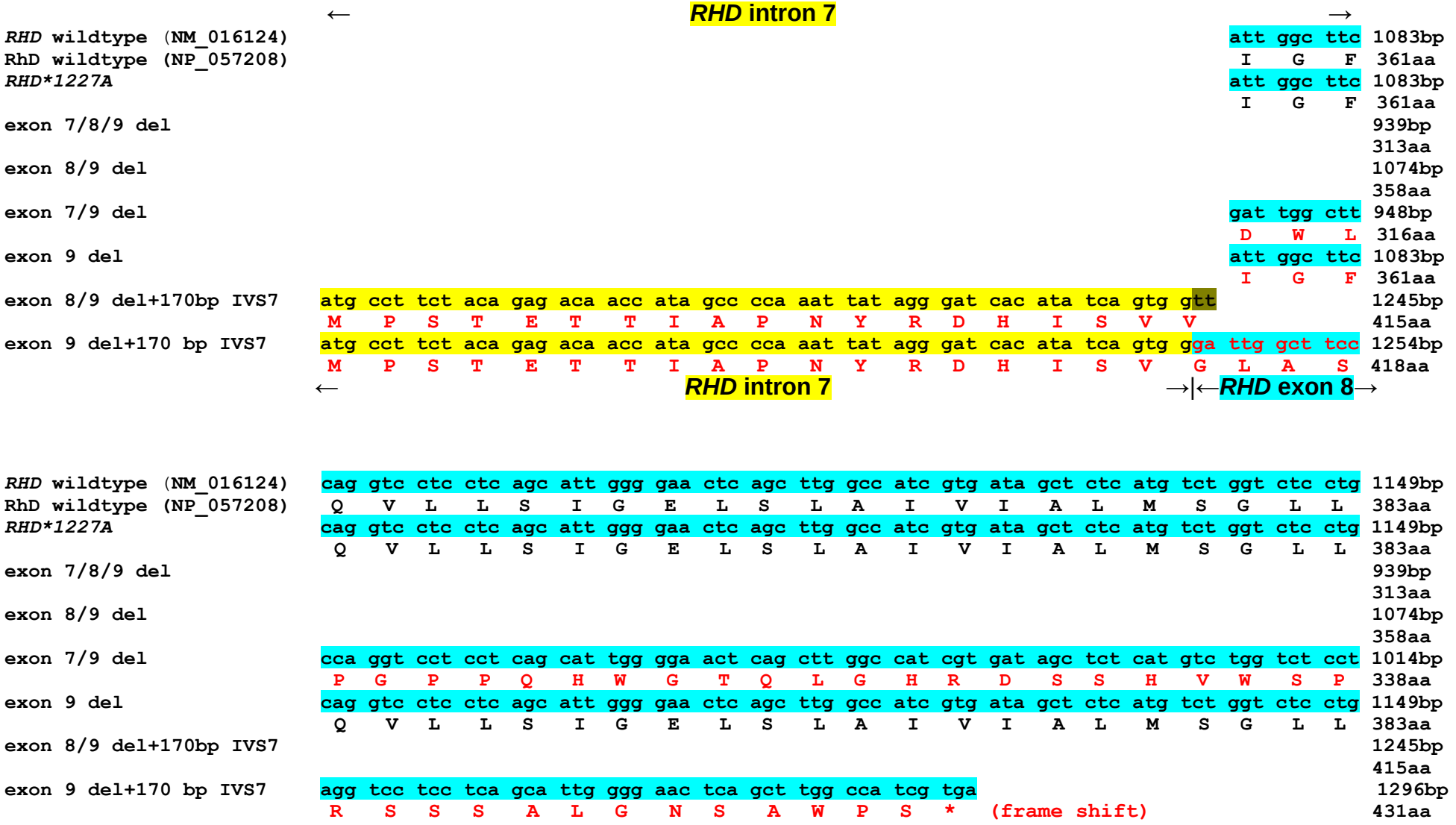
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I G F 361aa
exon 7/8/9 del
939bp
313aa
exon 8/9 del
1074bp
358aa
exon 7/9 del
gat tgg ctt 948bp
D W L 316aa
exon 9 del
att ggc ttc 1083bp
I G F 361aa
exon 8/9 del+170bp IVS7
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M P S T E T T I A P N Y R D H I S V V 415aa
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RHD*1227A
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Q V L L S I G E L S L A I V I A L M S G L L 383aa
exon 7/8/9 del
939bp
313aa
exon 8/9 del
1074bp
358aa
exon 7/9 del
cca ggt cct cct cag cat tgg gga act cag ctt ggc cat cgt gat agc tct cat gtc tgg tct cct 1014bp
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exon 9 del
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Q V L L S I G E L S L A I V I A L M S G L L 383aa
exon 8/9 del+170bp IVS7
1245bp
415aa
exon 9 del+170 bp IVS7
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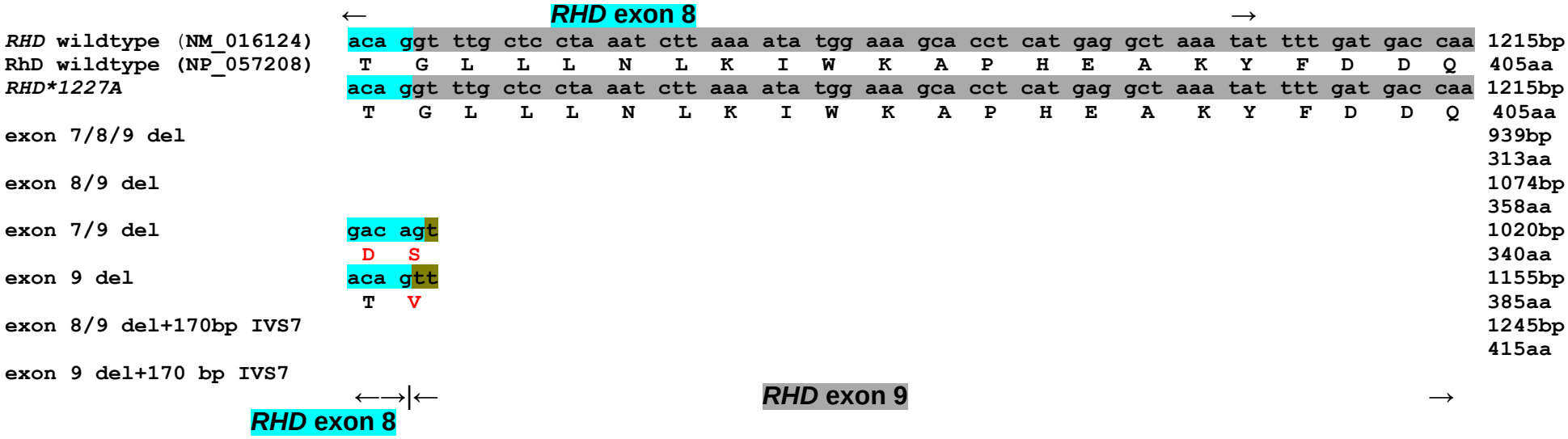
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RHD wildtype (NP_057208)
RHD*1227A
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T G L L L N L K I W K A P H E A K Y F D D Q 405aa
exon 7/8/9 del 939bp
313aa
exon 8/9 del 1074bp
358aa
exon 7/9 del gac agt 1020bp
D S 340aa
exon 9 del aca gtt 1155bp
T V 385aa
exon 8/9 del+170bp IVS7 1245bp
415aa
exon 9 del+170 bp IVS7
RHD exon 8
RHD exon 9
RHD*1227A
RHD wildtype (NM_016124)
gtt ttc tgg aag ttt cct cat ttg gct gtt gga ttt taa 1254bp
V F W K F P H L A V G F * 417aa
RHD wildtype (NP_057208)
RHD*1227A
gtt ttc tgg aaA ttt cct cat ttg gct gtt gga ttt taa 1254bp
V F W K F P H L A V G F * 417aa
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F P H L A V G F * (truncated) 321aa
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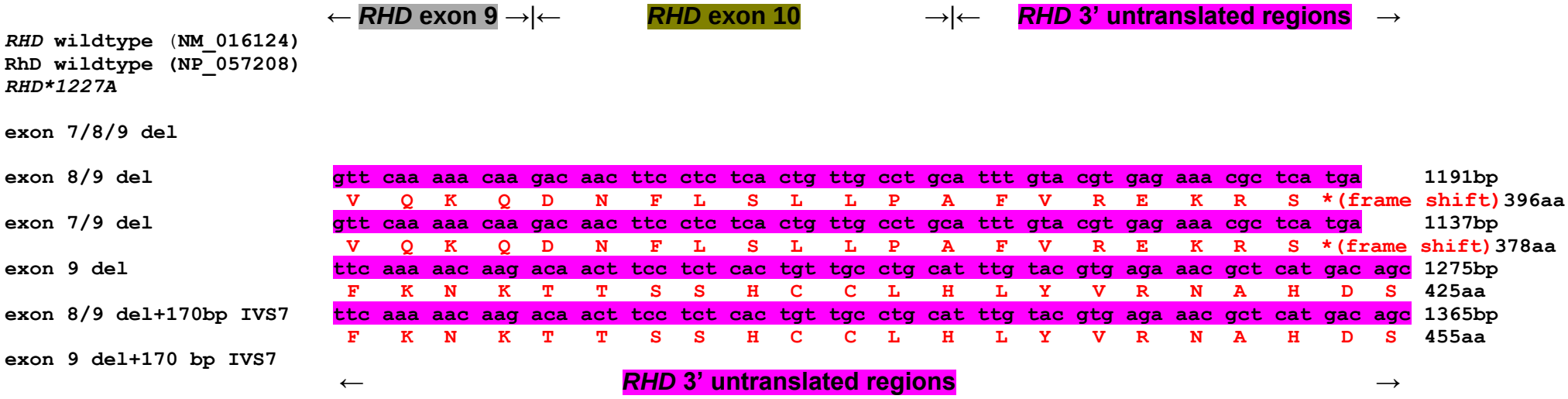
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RhD wildtype (NP_057208)
RHD*1227A
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exon 8/9 del
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exon 7/9 del
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exon 8/9 del+170bp IVS7
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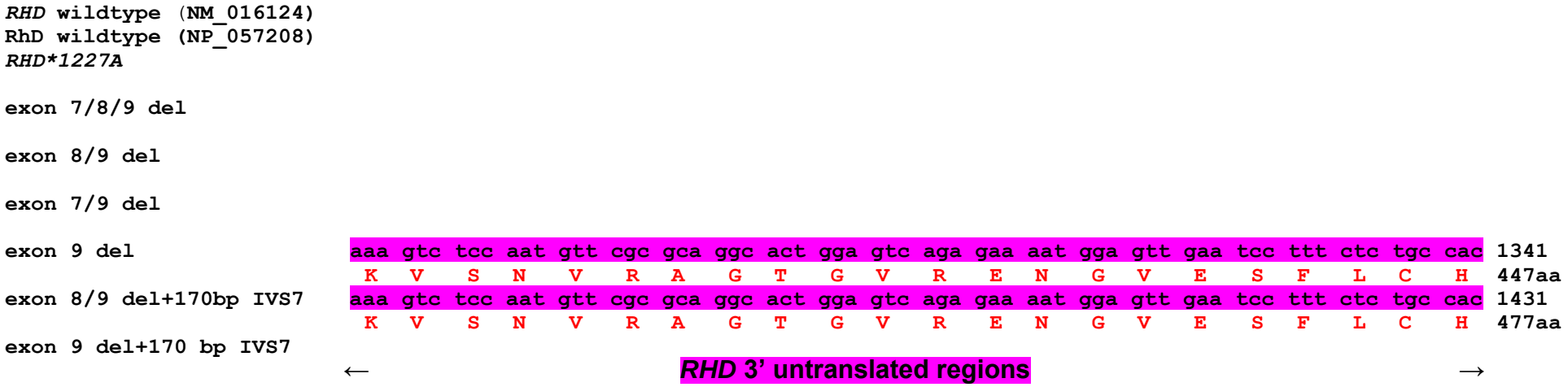
exon 9 del+170 bp IVS7
← RHD 3' untranslated regions →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RhD wildtype (NP_057208)
RHD*1227A
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exon 8/9 del
exon 7/9 del
exon 9 del
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K V S N V R A G T G V R E N G V E S F L C H 447aa
exon 8/9 del+170bp IVS7
aaa gtc tcc aat gtt cgc gca ggc act gga gtc aga gaa aat gga gtt gaa tcc ttt ctc tgc cac 1431
K V S N V R A G T G V R E N G V E S F L C H 477aa
exon 9 del+170 bp IVS7
← RHD 3' untranslated regions →

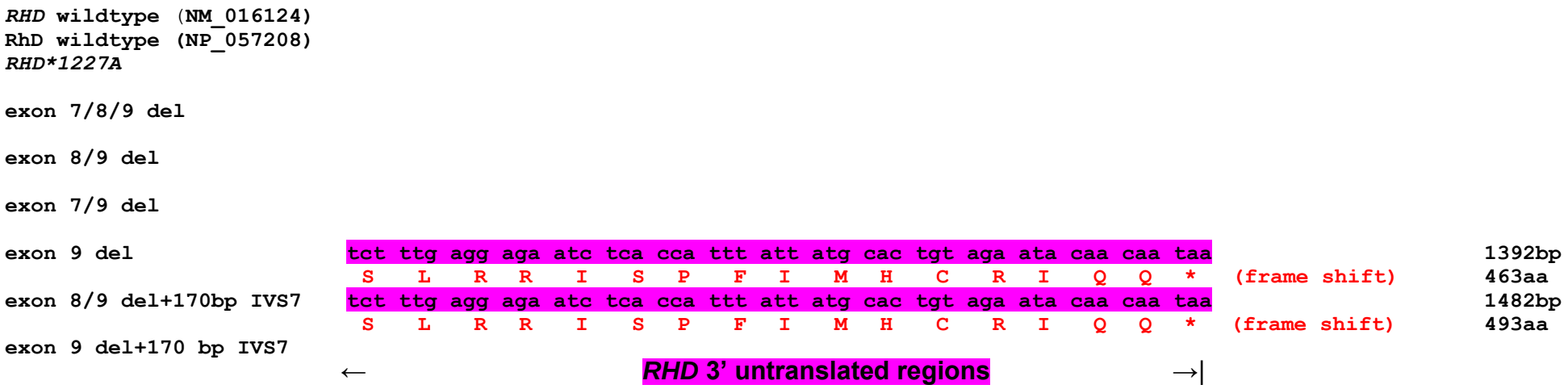

Figure S8. Comparison of the nucleotide and deduced amino acids sequences of the major types of *RHD* variant transcripts identified in Asian-type DEL individuals

Nucleic acid sequences of exon 1-5 were not shown since they are all identical. The nucleic acid sequences of exon 7, intron 7, exon 8, exon 9, exon 10 and 3' untranslated regions are highlighted in different colors. The full-length *RHD* transcript carrying the c.1227A variant (*RHD*1227A*) was identified in Asian-type DEL individuals by nanopore sequencing, while other six previously reported *RHD* spliced transcripts, including exon 9 deletion (NM_001282870), exon 8/9 deletion (NM_001282869), exon 7/9 deletion (NM_001282868), exon 7/8/9 deletion (NM_001127691), exon 9 deletion with insertion of 170 bp of intron 7 (NM_001282872), and exon 8/9 deletion with insertion of 170 bp of intron 7 (NM_001282871), were also identified.
Comparison of the deduced amino acid sequences (listed as one-letter amino acid code) transcribed by the major *RHD* transcripts identified in Asian-type DEL individuals was also shown. The deduced amino acid sequences that were different from the wild-type sequences (NP_057208) due to frameshift mutations are highlighted in red.

Table S1. Results *of RHD* genotyping for 541 patients and 1036 Chinese pregnant women with DEL phenotype

Individuals with DEL phenotype	Number of samples	Frequency	***RHD*_Allele 1**	***RHD*_Allele 2**
Patients	464	85.8%	*RHD*1227A*	-
(n=541)	50	9.2%	*RHD*1227A*	*RHD*1227A*
	12	2.2%	*RHD*1227A*	*RHD*D-CE(2-9)-D*
	3	0.6%	*RHD*1227A*	*RHD*711delC*
	3	0.6%	*RHD*1227A*	*RHD*D-CE(2-10)*
	1	0.2%	*RHD*1227A*	*RHD*CE(1-9)-D*
	1	0.2%	*RHD*1227A*	*RHD*D-CE(2-7)-D*
	1	0.2%	*RHD*1227A*	*RHD*D-CE(4-9)-D*
	1	0.2%	*RHD*1227A*	*RHD*D-CE(4-7)-D*
	1	0.2%	*RHD*1227A*	*RHD*421delG* *
	1	0.2%	*RHD*1227A*	*RHD*IVS5+2G* *
	1	0.2%	*RHD*1227A*	*RHD*1155C* *
	1	0.2%	*RHD*D-CE(4-7)-D*	-
	1	0.2%	*RHD*222A* *	-
Pregnant women	863	83.1%	*RHD*1227A*	-
(n=1,036)	102	9.8%	*RHD*1227A*	*RHD*1227A*
	53	5.1%	*RHD*1227A*	*RHD*D-CE(2-9)-D*
	6	0.6%	*RHD*1227A*	*RHD*711delC*
	2	0.2%	*RHD*1227A*	*RHD*D-CE(2-7)-D*
	1	0.1%	*RHD*1227A*	*RHD*D-CE(2-10)*
	4	0.4%	*RHD*1227A*	*RHD*CE(1-9)-D*
	1	0.1%	*RHD*1227A*	*RHD*680_684delTGCTG* *
	1	0.1%	*RHD*744T*	-
	1	0.1%	*RHD*1222C*	-
	1	0.1%	*RHD*761T* *	-
	1	0.1%	*RHD*1227delG* *	-

* Novel *RHD* alleles identified.

For the total of 1,571 Asian-type DEL pregnant women and patients identified, RhCE typing data showed that they were all positive for RhC antigen (Ccee, n = 1,210; CCee, n = 329; CcEe, n = 25; CCEe, n = 7).

Table S2. Alloantibodies against RBCs antigens detected in the patients and pregnant women with true D– and Asian-type DEL phenotypes

Individuals with serological apparent D– phenotype *		true D– patients §			Asian-type DEL patients §		
		Numbers	Alloantibodies		Numbers	Alloantibodies	
			Types	Numbers		Types	Numbers
Patients †			Anti-D	63		anti-Mi(a)	2
(n=2,011)			Anti-D+anti-C	6		Anti-E	1
			Anti-Mi(a)	4		Anti-M	1
		1,470 (73.1%)	Anti-D+anti-E	1	539 (26.8%)	anti-Jk^{a}	1
			Anti-E	1		anti-Chido/Rodgers	1
			Anti-M	1		-	-
			anti-Jk^{a}	1		-	-
		Total		77			6
	Pregnancy history ‡	**true D– pregnant women**			**Asian-type DEL pregnant women**		
Pregnant women	G1	1,123	Anti-D	4	394	UD	UD
			Anti-D+anti-C	1		UD	UD
(n=4,045)			Anti-M	2		UD	UD
	G2	757	Anti-D	20	262	Anti-M	1
			Anti-D+anti-C	2		UD	UD
			Anti-M	1		UD	UD
	G3	324	Anti-D	7	113	UD	UD
			Anti-D+anti-C	5		UD	UD
			Anti-Mi(a)	1		UD	UD
			Anti-cE + anti-Jk^{b}	1		UD	UD
	G4	121	Anti-D	3	43	UD	UD
			Anti-C	1		UD	UD
			Anti-E	1		UD	UD
	G5	46	Anti-D	3	12	UD	UD
	G6	8	Anti-D+anti-C	1	5	UD	UD
	G≥7	5	UD	UD	4	UD	UD
	unknown	625	Anti-D	29	199	Anti-Mi(a)	1

		Anti-D+anti-E	2		Anti-Le[a]	1
		Anti-D+anti-C	2		UD	UD
		Anti-E	2		UD	UD
		Anti-M	1		UD	UD
Total	3,009 (74.4%)		89	1,032 (25.5%)		3

* Among them, a total of 6 individuals, who did not produce alloantibodies against RBCs antigens, were identified with DEL phenotype carried *RHD* variant alleles except for Asian-type DEL allele.

† The patients are the inpatients applying for blood group testing before transfusions in the clinic.

‡ Only the gestational (G) time but no production (P) time was shown. For example, G5P0, G5P1, G5P2, G5P3, and G5P4, were all involved in the group of G5.

§ Before the trial, the difference in alloanti-D rate between the true D– and Asian-type DEL patient groups was statistically significant ($P < 0.001$, $\chi 2$ test, two sided).

Table S3. Information of 54 Asian-type DEL recipients who received D+ RBC transfusion

Recipients		Age (years)	Gender	Pregnancy history	Diagnosis	Blood group typing			Transfused D+ RBCs units †	Alloantibody test after D+ RBCs transfusion		Effectiveness of transfusion (Hb changes)
						DEL Serological A/E testing	RhCE phenotype	*RHD* genotypes		Observation time (days) ‡	Results	
1	DEL-18	76	M	-	Abdominal surgery	2+	Ccee	*RHD*1227A*	4U; 2U (2 days)	28; 344	Neg	4U: 141g/L→ 76g/L (Intraoperative blood loss 2000ml); 2U (2 days): 76g/L→87g/L
2	DEL-76	72	M	-	Coronary heart disease	2+	CCee	*RHD*1227A/1227A*	2U	6; 192	Neg	124g/L→128g/L (Intraoperative blood loss unkown)
3	DEL-168	64	F	G4P4	Ovarian cancer	2+	Ccee	*RHD*1227A*	2U	167	Neg	106g/L→103g/L (Intraoperative blood loss 500ml)
4	DEL-177	30	F	G5P5	Hepatic metastasis of colonic carcinoma	2+	Ccee	*RHD*1227A*	4U	171	Neg	62g/L→78g/L
5	DEL-211	63	M	-	Liver cancer	3+	Ccee	*RHD*1227A*	4U; 2U (5 days)	230	Neg	4U: 75g/L→ 81g/L; 2U (5 days): 69g/L→78g/L
6	DEL-230	52	F	G2P2	Uremia	+	Ccee	*RHD*1227A*	1U	206; 224; 487	Neg	55g/L→60g/L
7	DEL-231	66	M	-	Lung cancer	+	Ccee	*RHD*1227A*	2U	222	Neg	57g/L→83g/L

8	DEL-234	105 days	M	-	Extremely premature infant	+	Ccee	*RHD*1227A*	0.5U	693	Neg	96g/L→92g/L (Blood loss unkown)
9	DEL-265	49	M	-	Liver cancer	2+	Ccee	*RHD*1227A*	4U; 4U (22 days); 2U (23 days); 2U (24 days)	26; 27; 28; 257	Neg	4U: 124g/L→ 133g/L 4U (22 days), 2U (23 days): 62g/L →79g/L 2U (24 days): 86g/L→122g/L
10	DEL-368	55	M	-	Upper gastrointestinal bleeding	3+	Ccee	*RHD*1227A*	2U； 3.5U (372 days); 2U (373 days)	209; 386	Neg	2U: 64g/L→ 80g/L 3.5U (372 days): 35g/L→48g/L 2U (373 days): 48g/L→65g/L
11	DEL-469	76	M	-	Upper gastrointestinal bleeding	3+	Ccee	*RHD*1227A*	2U	165	Neg	ND **
12	DEL-522	18	M	-	Scoliosis	2+	Ccee	*RHD*1227A*	8U	184; 281	Neg	152g/L→138g/L (Intraoperative blood loss unkown)
13	DEL-527	46	F	G2P2	Gastric carcinoma	1+	Ccee	*RHD*1227A*	4U; 4U (6 days)	92	Neg	4U: 76g/L→ 97g/L; 4U (6 days): 97g/L→111g/L (Intraoperative blood loss 100ml)
14	DEL-572	52	F	G2P2	Humeral fractures	2+	Ccee	*RHD*1227A*	2U	195	Neg	93g/L→104g/L (Intraoperative

												blood loss unkown)
15	DEL-574	54	M	-	Femoral fracture	2+	Ccee	*RHD*1227A*	2U; 4U (5 days); 2U (5 days)	57	Neg	2U: 63g/L→69g/L; 2U (5 days): 73g/L→63g/L (Intraoperative blood loss 400ml)
16	DEL-628	4	M	-	Neuroblastic tumor	3+	Ccee	*RHD*1227A*	2U; 1U (21 days); 2U (35 days); 1U (59 days); 2U (66 days); 2U (247 days); 2U (254 days); 2U (282 days)	19; 63; 244; 289	Neg	2U: 54g/L→90g/L; 1U (21 days): 57g/L→69g/L; 2U (35 days): 61g/L→93g/L; 1U (59 days): 44g/L→63g/L; 2U (66 days): 57g/L→95g/L; 2U (247 days): 24g/L→58g/L
17	DEL-629	28	M	-	Gastrointestinal bleeding	3+	Ccee	*RHD*1227A*	4U; 4U (86 days); 4U (89 days)	84; 444	Neg	4U: 71g/L→104g/L; 4U (86 days): 59g/L→94g/L; 4U (89 days): 95g/L→108g/L
18	DEL-630	49	M	-	Femoral head necrosis	3+	Ccee	*RHD*1227A*	4U	152	Neg	147g/L→150g/L (Intraoperative blood loss 600ml)

19	DEL-633	49	M	-	Subarachnoid hemorrhage	3+	CCee	*RHD*1227A/D-CE(2-9)-D*	2U	302	Neg	128g/L→111g/L (Intraoperative blood loss 400ml)
20	DEL-679	13	M	-	Neuroblastoma	2+	Ccee	*RHD*1227A*	1U; 2U (6 days); 2U (24 days); 2U (31 days); 2U (46 days); 1U (818 days); 1U (840 days); 1U (1168 days); 2U (1176 days); 2U (1187 days); 2U (1196 days); 2U (1207 days); 2U (1245 days); 2U (1256 days); 1U (1345 days); 1U (1346 days); 2U（1355 days)	1168; 1345; 1354	Neg	2U (31 days): 53g/L→81g/L 1U (1168 days): 43g/L→51g/L
21	DEL-797	50	F	G4P2	Abnormal uterine bleeding; Severe anemia	3+	Ccee	*RHD*1227A*	2U	179	Neg	59g/L→85g/L
22	DEL-872	37	M	-	Chronic renal failure; Anemia	3+	Ccee	*RHD*1227A*	2U; 2U (2 day); 2U (3 days); 2U (4 days); 2U (5 days); 2U (6 days)	1;2;3;4;5; 449	Neg	2U; 2U (2 day); 2U (3 days): 49g/L→64g/L

23	DEL-890	78	M	-	Chronic anemia	3+	CCee	*RHD*1227A/1227A*	2U; 2U (63 days); 2U (73 days)	61; 70; 192	Neg	ND **
24	DEL-909	44	M	-	Heart valvular disease	2+	Ccee	*RHD*1227A*	2U	104	Neg	111g/L→105g/L (Intraoperative blood loss unkown)
25	DEL-989	53	F	G3P3	Meningioma	3+	Ccee	*RHD*1227A*	2U	397	Neg	ND **
26	DEL-994	26	M	-	Pituitary adenoma	2+	Ccee	*RHD*1227A*	2U	43	Neg	ND **
27	DEL-1025	49	F	G2P2	Gallstone	3+	CCee	*RHD*1227A*	4U	32	Neg	92g/L→106g/L
28	DEL-1091	66	M	-	Rectal cancer	2+	Ccee	*RHD*1227A*	4U	332	Neg	60g/L→81g/L (Intraoperative blood loss 50ml)
29	DEL-1117	86	M	-	Rectal cancer	3+	CCee	*RHD*1227A/1227A*	2U	831	Neg	ND **
30	DEL-1268	80	M	-	Tumor with severe anemia	3+	CCee	*RHD*1227A/D-CE(2-9)-D*	2U; 2U (4 days); 4U (696 days)	4; 14; 27;155; 695; 704	Neg	4U (696 days): 58g/L→92g/L
31	DEL-1324	75	F		Femoral neck fracture	3+	Ccee	*RHD*1227A*	2U; 2U (3 days)	3, 86	Neg	2U, 2U (3 days): 91g/L→81g/L (Intraoperative blood loss 200ml)
32	DEL-1329	40	M	-	Lung cancer	3+	Ccee	*RHD*1227A*	6U	69	Neg	112g/L→119g/L (Intraoperative blood loss unkown)
33	DEL-1401	70	M	-	Lumbar spinal stenosis	3+	Ccee	*RHD*1227A*	2U	803	Neg	ND **

34	DEL-1420	62	M	-	Gastrorrhagia	3+	Ccee	*RHD*1227A*	2U; 2U (2 days); 1.5U (5 days)	1; 2; 5; 556	Neg	2U: 59g/L→68g/L; 2U (2 days): 68g/L→76g/L; 1.5U (5 days): 67g/L→75g/L
35	DEL-1436	47	F	G1P1	Lung cancer with brain metastasis	2+	Ccee	*RHD*1227A*	4U; 4U (24 days); 4U (114 days)	24; 114	Neg	4U: 62g/L→86g/L; 4U (24 days): 78g/L→104g/L; 4U (114 days): 68g/L→118g/L
36	DEL-1487	72	M	-	Kidney neoplasms	3+	CCee		2U	506	Neg	114g/L→125g/L
37	DEL-1659	6	M	-	Neuroblastoma		Ccee	*RHD*1227A*	1U; 1U (7 days); 2U (8 days); 2U (41 days); 1 U (83 days)	7; 8; 41; 83	Neg	1U: 55g/L→81g/L; 1U (7 days): 33g/L→31g/L; 2U (41 days): 44g/L→70g/L
38	DEL-1720	78	M	-	Fracture	3+	Ccee	*RHD*1227A*	4U	2; 6; 260	Neg	91g/L→85g/L (Intraoperative blood loss 400ml)
39	DEL-1900	62	M	-	Severe pneumonia	3+	Ccee	*RHD*1227A*	2U	10; 329	Neg	69g/L→81g/L

40	DEL-1960	49	F	-	Evans syndrome	ND *	Ccee	*RHD*1227A*	2U; 2U (2 days); 2U (3 days); 2U (9 days); 4U (17 days); 2U (27 days); 2U (130 days)	1; 8; 15; 26; 37; 129; 133	Anti-Mi(a)	2U: 50g/L→ 59g/L 2U (2 days): 59g/L→64g/L 2U (3 days): 64g/L→65g/L (Intraoperative blood loss unkown) 2U (9 days): 61g/L→65g/L 4U (17 days): 48g/L→73g/L 2U (27 days): 56g/L→58g/L 2U (130 days): 63g/L→69g/L
41	DEL-1982	67	M	-	Gangrene; Anemia	3+	Ccee	*RHD*1227A*	2U; 2U (84 days); 2U (85 days); 4U (96 days); 4U (102 days)	83; 95; 101	Neg	2U (84 days): 75g/L→94g/L
42	DEL-2159	58	F	G2P2	Ovarian cancer	3+	Ccee	*RHD*1227A*	2U; 2U (1 day); 4U (5 days); 1.5U (89 days); 1U (93 days)	5; 40; 88; 92	Neg	2U: 45g/L→ 60g/L 2U (1 day): 60g/L→75g/L 4U (5 days): 80g/L→100g/L
43	DEL-114	64	F	G0P0	Cardiosurgery	ND *	Ccee	*RHD*1227A*	2U; 4U (6 days)	7 §	Neg	2U: 70g/L→ 83g/L; 4U (6 days): 76g/L→99g/L

44	DEL-792	62	F	G3P3	Liver cancer; Hemobilia	2+	Ccee	*RHD*1227A*	2U; 8U (13 days)	12 §	Neg	2U: 60g/L→ 74g/L
45	DEL-1074	36	M	-	Heart failure	3+	Ccee	*RHD*1227A*	2U; 2U (12 days); 2U (14 days);	12; 14 §	Neg	2U: 57g/L→ 73g/L; 2U: (12 days): 49g/L→50g/L; 2U: (14 days): 40g/L→41g/L
46	DEL-1906	54	F	-	Spinal malignancy	3+	Ccee	*RHD*1227A*	10U	19 §	Neg	127g/L→94g/L (Intraoperative blood loss 900ml)
47	DEL-86	61	F	ND	Lupus nephritis	2+	CcEe	*RHD*1227A/421delG*	4U		ND §	
48	DEL-113	86	M	-	Hemorrhagic shock	ND *	Ccee	*RHD*1227A*	2U		ND‖	
49	DEL-259	81	F	ND	Bone fistula	+	CCee	*RHD*1227A*	4U		ND §	
50	DEL-315	91	F	ND	Fracture	2+	CCee	*RHD*1227A/D-CE(2-8)-D*	4U		ND ¶	
51	DEL-343	60	F	G5P5	Cervical cancer	2+	CCee	*RHD*1227A*	2U		ND‖	
52	DEL-472	47	F	ND	Anemia	3+	Ccee	*RHD*1227A*	4U		ND ¶	
53	DEL-662	88	M	-	Gastrointestinal bleeding	3+	Ccee	*RHD*1227A*	2U		ND ¶	
54	DEL-1665	49	M	-	Gastrointestinal bleeding	2+	Ccee	*RHD*1227A*	4U		ND §	

* Adsorption and elution testing was not conducted because of positive DAT (3+) identified in the patients.
† In the bracket, the days for the next time of D+ blood transfusion were counted from the first day to receive D+ blood transfusion.
‡ The exact days were counted from the first day to receive D+ blood transfusion.
§ No further sample was available for follow-up antibody screening as the patient passed away.
‖ The follow-up data after D+ blood transfusion was not available for noncooperative recipients.

¶ The patients were out of touch for follow-up.
** Improvement of anemia symptoms was observed.
Abbreviation: A/E testing: Adsorption/elution testing; Neg: Negative results for antibody screening after receiving D+ blood transfusion; ND: Not detected.

Table S4. The total reads and mapping rate of Nanopore sequencing to *RHD* transcripts in eight Asian-type DEL individuals and one D+ control

Sample ID	*RHD* genotypes	Total Reads	Mapped Reads	Mapping rate
G707	*RHD*1227A/1227A*	245790	240085	97.68%
G927	*RHD*1227A/01N.01*	213241	208556	97.80%
G1191	*RHD*1227A/1227A*	223884	215382	96.20%
G1493	*RHD*1227A/01N.01*	285986	274253	95.90%
G2076	*RHD*1227A/1227A*	3591741	3550457	98.85%
G2245	*RHD*1227A/1227A*	217099	212156	97.72%
G2839	*RHD*1227A/01N.01*	260482	256434	98.45%
G3068	*RHD*1227A/01N.01*	238167	232314	97.54%
D+ control	*RHD*01/01*	107561	105769	98.33%

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