Rode Kruis Vlaanderen

Challenging inno-train
Comparison of SSP and NGS in RBC genotyping

Vicky Van Sandt

05-11-2024

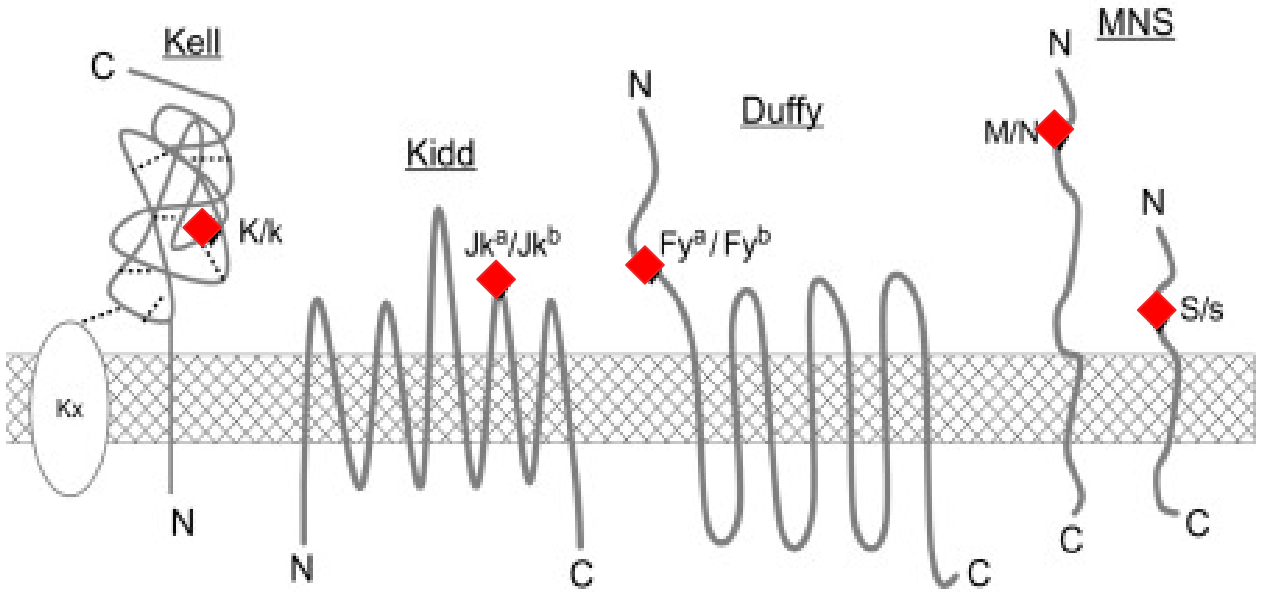
Red Cross Flanders - BL - HILA

Red Blood Cell Antigen Genotyping

- DAT +	vERYfy
- reagents/monoclonals are not available	vERYfy eXtend
- Therapeutic interferrence	
- Transfusion	
- Serological weak or discrepant reactivities	Weak D, Partial D,
Variant alleles	CDE eXtend
- RHD zygosity	RH TYPE

+/- 2000 tests per year
= all SNP tests
Test most relevant polymorphisms
Most likely allele

Most RBC antigens are SNPs single nucleotide polymorfisms:



Standard:
Kell – K, k
Kidd – Jka, Jkb
Duffy – Fya, Fyb, FyX, FyGATA
MNS – M, N, S, s,Uvar
Rh – C, c, E, e
Dombrock – Doa,Dob

HFA:
Kell - Kpa, Kpb Jsa, Jsb
Colton – Coa, Cob
Lutheran – Lua, Lub
Diego – Dia, Dib
Cartwright – Yta, Ytb
Knops – Kna, Knb

Reaktions-Nr. / *Reaction no.*			1	2	3	4	5	6	7	8	9	10	11	12 ◆	13	14	15
PCR-Produkt (Größe in bp) / *PCR product (size in bp)*			134	148	118	135	132	132	120	673	184	132	188	107	117	128	140
RHD Exons * Mix 8: Intron 7/ Exon 8		Haplotyp *Haplotype*	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_{78}*	D_9	D_{10}	D_2	D_6	D_7	D_7	D_8
Spezifität / *Specificity*			Standard RHD														
RHD*01	RHD pos.		+	+	+	+	+	+	+	+	+	+					
			RHD negative														
RHD*01N.01	RHD neg. cc																
RHD*01N.01	RHD neg. Cc oder / or CC			+													
			RHD Varianten / *RHD Variants*														
RHD*DII	RHD*02	CDe	+	+	+	+	+	+	+	+	+	+				+	
RHD*DIIIc	RHD*03.03	CDe	+	+		+	+	+	+	+	+	+					
RHD*DIIIb[n.t.]	RHD*03.02[n.t.]	cDe	+		+	+	+	+	+	+	+	+					
RHD*DIVa[n.t.]	RHD*04.01[n.t.]	cDe	+	+		+	+	+			+	+					
RHD*DIVb,*DIV.5[n.t.]	RHD*04.08, *04.05[n.t.]	cDE	+	+	+	+	+	+				+					
RHD* DIV.3	RHD*04.03	CDe	+	+	+	+	+					+					
RHD*DIV.4	RHD*04.04	CDe	+	+	+	+	+	+		+	+	+				+	
RHD*DV.1 - *DV.10, RHD*DBS1,*DBS2	RHD*05.01 - *05.10, RHD*13.01,*13.02	CDe	+	+	+	+		+	+	+	+	+					
RHD*DVI.1	RHD*06.01	cDE	+	+	+			+	+	+	+	+					
RHD*DVI.2	RHD*06.02	CDe	+	+	+				+	+	+	+					
RHD*DVI.3	RHD*06.03	CDe	+	+					+	+	+	+					
RHD*DVI.4	RHD*06.04	Cde	+	+				+	+	+	+	+					
RHD*DVII.1	RHD*07.01	CDe	+	+	+	+	+	+	+	+	+	+	+				
RHD*DAR1.00 (weak D 4.2)	RHD*08.01.00	cDe	+	+	+	+	+	+	+		+	+					
RHD*DAR2.00,*DAR2.01	RHD*08.02.00, *08.02.01	cDe	+	+	+	+		+	+		+	+					
RHD*DAU0 - *DAU3, *DAU8 - *DAU15	RHD*10.00-*10.03, *10.08 - *10.15	cDe	+	+	+	+	+	+	+	+	+	+					+
RHD*DAU4, *DAU6[n.t.], *DAU5.01[n.t.]	RHD*10.04, *10.06[n.t.], *10.05.01 [n.t.]	cDe	+	+	+	+		+	+	+	+	+					+
RHD*DBT1	RHD*14.01	CDe	+	+	+	+					+	+					
RHD*DBT2[n.t.]	RHD*14.02[n.t.]	CDe	+	+	+	+						+					
RHD*DFR1 -*DFR3, *DFR5 ◆ oder / or RHD* Ψ ◆	RHD*17.01 - *17.03, *17.03 oder / or RHD*08N.01		+	+	+		+	+	+	+	+	+					
RHD*DHMI	RHD*18	cDE	+	+	+	+	+	+	+	+	+	+		+			
RHD*DNB	RHD*25	CDe	+	+	+	+	+	+	+	+	+	+			+		
RHCE*ce-D(5)-ce (RH:33 (DHAR+))	RHCE*01.22	cDe					+										
RHD*CE(1-8)-D	RHD*01N.02	cDE										+					
RHD*D-CE(2-8)-D	RHD*01N.03	CDe	+	+								+					
RHD*D-CE(3-7)-D	RHD*01N.05	CDe oder / or (C)de*	+	+						+	+	+					
RHD*D-CE(4-7)-RHD1	RHD*01N.07	cDE	+	+	+						+	+					
RHD*D-CE(4-7)-RHD2	RHD*01N.07	cDE	+	+	+					+	+	+					
RHD*D-CE(8-9)-D		Cde	+	+	+	+	+	+	+			+					
RHD(delEx9) ◆		CDe	+	+	+	+	+	+	+	+		+					
RHD*D-CE(10) ◆		Cde	+	+	+	+	+	+	+	+	+						
RHD(Q41X)[n.t.]	RHD*01N.09	Cde		+	+	+	+	+	+	+	+	+					
DC3, [DFW, DHR, DIM, DNU] [n.t.]		diverse, various	+	+	+	+	+	+	+	+	+	+					

n.t. / [ ] n.t. = not tested currently

Genotyp / *Genotype*		Phänotyp / *Phenotype*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

RHCE: CDE eXtend

Single Nucleotide Polymorphisms RHCE: 4 → 30

GOAL: discriminate the most important/relevant RHCE genotypes/phenotypes.

RBC-FluoGene CDE eXtend

CE IVD

LOT R920323

2025-02

Spezifitätentabelle / *Specificity table*

Der ISBT Phänotyp ist bei den RHCE varianten Allelen zur besseren Übersichtlichkeit gekürzt. Bei Bedarf ist die originale ISBT Tabelle zu beachten.

For reasons of clarity, the ISBT phenotype of the RHCE variant alleles was shortened. Please refer to the original ISBT table.

Blood Group System	Reaktion / Reaction	Position	Spezifität / Specificity	SNP	ISBT Allelname / Allele name	ISBT Phänotyp / Phenotype	RBC marker [Color]	IC marker [color]
RHCE	c-1, c-2[a]	F1, F3	c	201A 307C	RHCE*01 (RHCE*ce)RHCE*03 (RHCE*cE)	RH:4 (c)	2	3
	C-1, C-2	G1, G3	C	i2-109 bp ins	RHCE*02 (RHCE*Ce)RHCE*04 (RHCE*CE)	RH:2 (C)	1	3
	Cw-1, Cw-2	E1, E3	C^w	(-132G) 122A>G	RHCE*02.08.01 (RHCE*Ce.08.01, RHCE*CeCW) RHCE*02.08.02 (RHCE*Ce.08.02, RHCE*CeNR)	RH:8 (C^w+)	2	3
	e-1, e-2[b]	F1, F3	e	676G (787A)	RHCE*01 (RHCE*ce)RHCE*02 (RHCE*Ce)	RH:5 (e)	1	3
	E-1, E-2[c]	G1, G3	E	676G>C (787A)	RHCE*03 (RHCE*cE)RHCE*04 (RHCE*CE)	RH:3 (E)	2	3
	CE-733G-1, CE-733G-2[d]	A3, A4	RHCE-733G	733C>G (i5+170G)	RHCE*01.04 RHCE*01.06.02/.03 RHCE*01.20.01 RHCE*01.20.02 RHCE*01.20.03 RHCE*01.20.04 RHCE*01.20.05 RHCE*01.20.06 RHCE*01.20.07 RHCE*01.20.08 RHCE*01.20.09 RHCE*01.20.10, *01.20.11, *01.20.12 RHCE*01.20.13 RHCE*01.22 RHCE*01.37 RHCE*02.04 RHCE*02.30 RHCE*02.40 RHCE*03.03.02	RH:10,-20 (V+^{w}VS-), RH:-19 (hrS-) RH:10,20 (V+VS+), RH:31 (hrB+ very weak to neg) RH:10,20 (V+VS+), RH:-31 (hrB-) RH:-10,20 (V-VS+), RH:-31 (hrB-) RH:10,20 (V+VS+), RH:-31 (hrB-) RH:-10,20 (V-VS+), RH:-31 (hrB-) RH:43 (Crawford+), RH:20 (VS+), RH:-19 (hrS-),RH:-31 (hrB-) RH:-58 (CELO-) RH:10 (V+ weak to neg), RH:20 (VS+ weak to neg), RH:19 (hrS+ weak to neg), RH:31 (hrB+ weak to neg), RH:48,(JAL+), RH:-57 (CEST-) RH:10,20 (V+VS+), RH:-31 (hrB-) RH:10,20 (V+VS+), RH:31 (hrB+ weak) RH:9 (Cx+), RH:20 (VS+) RH:33 (DHAR+), RH:50 (FPPT+) RH:10 (V+), RH:20 (VS+) RH:-10 (V-)	2	3
	CE-1006T[e]	B4	RHCE-1006T	(i6-159T)	RHCE*01.20.03	RH:-10 (V-), RH:20 (VS+), RH:-31 (hrB-)	1	3

RHCE: which variants to want to discriminate?

RHCE alleles		CDE eXtend	SIKKELCEL Chang *et al.* 2020 n= 884	SIKKELCEL Chou *et al.* 2018 n= 587	SIKKELCEL Van Sandt *et al.* 2023 n = 43	(ISBT 004) RHCE blood group alleles v6.2-31-MAR-2022	NOTE
RHCE*01	**ce**	X	0,2010	0,2730	0,1944	**c.307C c.676G**	
RHCE*01.20.01	**ceVS.01**	X	0,1390	0,1430	0,1667	**c.733C>G**	
RHCE*02	**Ce**	X	0,1170	0,1360	0,1389	**c.48G>C** c.150C>T c.178C>A c.201A>G c.203A>G c.307C>T **c.676G**	
RHCE*01.01	**ce.01**	X	0,2270	0,1930	0,1111	**c.48G>C**	
RHCE*03	**cE**	X	0,1020	0,1000	0,0694	**c.307C c.676G>C**	
RHCE*01.20.03	**ceS**	X	0,0450	0,0330	0,0694	**c.48G>C c.733C>G c.1006G>T**	
RHCE*01.06.01	**ceAG**	X	0,4100	0,0430	0,0417	**c.254C>G**	
RHCE*01.20.09	**ceVS.09**	X	0,2700	0,0000	0,0417	**c.48G>C c.733C>G** c.941T>C	1
RHCE*01.04.01	**ceAR**	X	0,0006	0,0030	0,0417	**c.48G>C c.712A>G c.733C>G c.787A>G c.800T>A c.916A>G**	
RHCE*01.02.01	**ce.01.02**	X	0,0300	0,0180	0,0278	**c.48G>C c.1025C>T**	
RHCE*04	**CE**	X	0,0011	0,0000	0,0278	**c.48G>C** c.150C>T c.178C>A c.201A>G c.203A>G c.307C>T **c.676G>C**	
RHCE*02:10	**CeRN**	X	0,0000	0,0017	0,0278	c.505C>A c.509G>T c.514T>A c.544A>T **c.577A>G** c.594T>A c.602G>C	
RHCE*03.04	**cEIV**		0,0017	0,0000	0,0139	c.602G>C	2
RHCE*03.18	**cE.18**	X	0,0000	0,0000	0,0139	**c.48G>C**	
RHCE*02:09	**CeCX**	X	0,0000	0,0000	0,0139	**c.106G>A**	
RHCE*01.20.02	**ceVS.02**	X	0,0310	0,0270	0,0000	**c.48G>C c.733C>G**	
RHCE*01.07.01	**ceMO**	X	0,0110	0,0140	0,0000	**c.48G>C c.667G>T**	
RHCE*01.08	**ceBI**	X	0,0020	0,0030	0,0000	**c.48G>C c.712A>G c.818C>T c.1132C>G**	
RHCE*01.20.06	**ceCF**	X	0,0020	0,0009	0,0000	**c.48G>C c.697C>G c.733C>G**	
RHCE*01.05.01	**ceEK**	X	0,0017	0,0003	0,0000	**c.48G>C c.712A>G c.787A>G c.800T>A**	
RHCE*01.20.05	**ceVS.05**	X	0,0017	0,0000	0,0000	**c.733C>G c.1006G>T**	
RHCE*01.03		X	0,0006	0,0000	0,0000	**c.1025C>T**	
RHCE*01.20.04.02	**ceTI type 2**	X	0,0006	0,0009	0,0000	**c.48G>C** c.105C>T **c.733C>G** c.744T>C **c.1025C>T**	
RHCE*01.20.07	**ceJAL**	X	0,0006	0,0018	0,0000	**c.340C>T c.733C>G**	

ISBT tabel : [004 RHCE Alleles | The International Society of Blood Transfusion (ISBT) (isbtweb.org)]

Flemish population:

Mapped presence and frequency of RHCE alleles in a cohort of Sickle cell patients (Van Sandt *et al.* 2023).

Compared our population with bigger studies in US (Chou et al 2018, Chang et al. 2020).

RHCE variants with a frequency ≥ 0,001 (Chou *et al.*, 2018., Chang *et al.*, 2020, Van Sandt *et al.*, 2023)

Relevant SNPs

Mutant en wild type SNP: zygosity of variants.

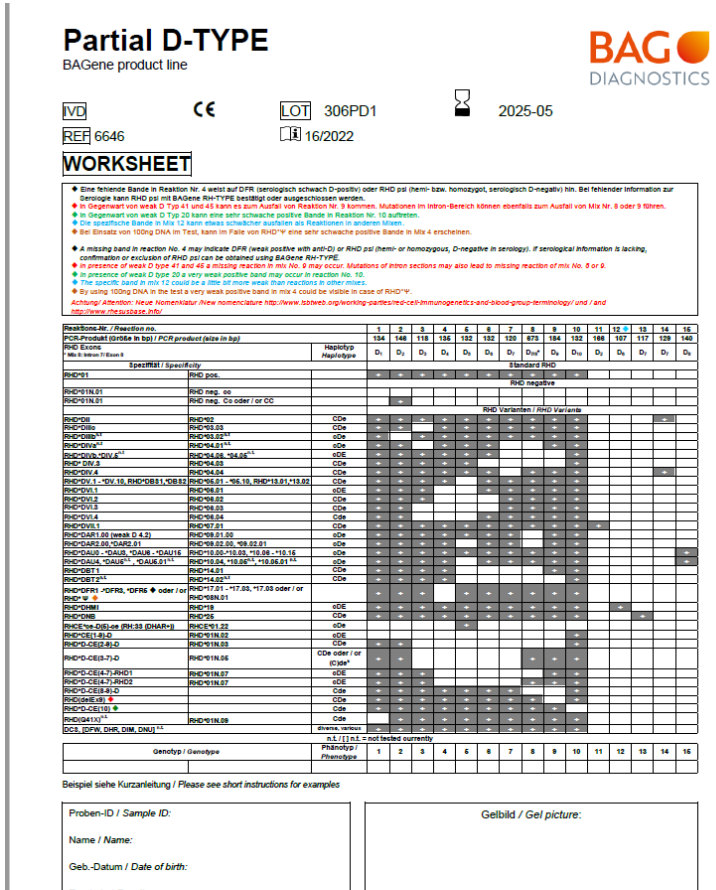
CDE eXtend

inno-train DIAGNOSTIK GMBH

CDE eXtend: RHD, which variants to want to discriminate?

Evaluation / comparison of the RHD pakket of

BAG **vs** **CDE eXtend**



Partial D-TYPE

BAGene product line

BAG DIAGNOSTICS

IVD CE LOT 306PD1 2025-05

REF 6646 16/2022

WORKSHEET

◆ Eine fehlende Bande in Reaktion Nr. 4 weist auf DFR (serologisch schwach D-positiv) oder RHD psi (hemi- bzw. homozygot, serologisch D-negativ) hin. Bei fehlender Information zur Serologie kann RHD psi mit BAGene RH-TYPE bestätigt oder ausgeschlossen werden.
◆ In Gegenwart von weak D Typ 41 und 45 kann es zum Ausfall von Reaktion Nr. 9 kommen. Mutationen im Intron-Bereich können ebenfalls zum Ausfall von Mix Nr. 8 oder 9 führen.
◆ In Gegenwart von weak D Typ 20 kann eine sehr schwache positive Bande in Reaktion Nr. 10 auftreten.
◆ Die spezifische Bande in Mix 12 kann etwas schwächer ausfallen als Reaktionen in anderen Mixen.
◆ Bei Einsatz von 100ng DNA im Test, kann im Falle von RHD*Y eine sehr schwache positive Bande in Mix 4 erscheinen.

◆ *A missing band in reaction No. 4 may indicate DFR (weak positive with anti-D) or RHD psi (hemi- or homozygous, D-negative in serology). If serological information is lacking, confirmation or exclusion of RHD psi can be obtained using BAGene RH-TYPE.*
◆ *In presence of weak D type 41 and 45 a missing reaction in mix No. 9 may occur. Mutations of intron sections may also lead to missing reaction of mix No. 8 or 9.*
◆ *In presence of weak D type 20 a very weak positive band may occur in reaction No. 10.*
◆ *The specific band in mix 12 could be a little bit more weak than reactions in other mixes.*
◆ *By using 100ng DNA in the test a very weak positive band in mix 4 could be visible in case of RHD*Y.*
Achtung! Attention: Neue Nomenklatur /New nomenclature http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/ und / and http://www.rhesusbase.info/

Reaktions-Nr. / Reaction no.			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PCR-Produkt (Größe in bp) / PCR product (size in bp)			154	148	118	136	132	132	120	673	184	132	166	107	117	129	140
RHD Exons * Mix 8: Intron 7/ Exon 8		Haplotyp / Haplotype	D_1	D_2	D_3	D_4	D_5	D_6	D_7	$D_{8/9}$*	D_9	D_{10}	D_{11}	D_8	D_7	D_7	D_8
Spezifität / Specificity			Standard RHD														
RHD*01	RHD pos.		+	+	+	+	+	+	+	+	+	+					
			RHD negative														
RHD*01N.01	RHD neg. oo																
RHD*01N.01	RHD neg. Co oder / or CC		-	-													
			RHD Varianten / RHD Variants														
RHD*DII	RHD*02	cDe	+	+	-	+	+	+	+	+	+	+				-	+
RHD*DIIIb	RHD*03.03	cDe	+	+	+	+	+	+	+	+	+	+					
RHD*DIIIb*[47]	RHD*03.22*[47]	cDe	+	-	+	+	+	+	+	-	+	+					
RHD*DIVa*[22]	RHD*04.01*[4,5]	cDe	+	+	+	+	+	+			-	+					
RHD*DIVb,*DIV.4*[47]	RHD*04.08, *04.6*[4,5]	cDE	+	+	+	+	+	+				+					
RHD* DIV.3	RHD*04.05	CDe	+	+	+	+	+	+				+					
RHD*DIV.4	RHD*04.04	CDe	+	+	+	+	+	+	+	+	+	+				+	
RHD*DIV.1 *DV.10, RHD*DBS1,*DBS3	RHD*05.01	cDE	+	+	+	+	-	+	+	+	+	+					
RHD*DVI.1	RHD*06.01	cDE	+	+	+	+		+	+	+	+	+					
RHD*DVI.2	RHD*06.02	CDe	+	+					+	+	+	+					
RHD*DVI.3	RHD*06.03	CDe	+	+					+	+	+	+					
RHD*DVI.4	RHD*06.04	Cde	+	+				+	+	+	+	+					
RHD*DVII.1	RHD*07.01	CDe	+	+	+	+	+	+	+	+	+	+	+				
RHD*DAR1.00 (weak D 4.3)	RHD*09.01.00	cDe	+	+	+	+	+	+				+					
RHD*DAR2.00,*DAR2.01	RHD*09.02.00, *09.02.01	cDe	+	+	+	+	+	+	+	+	+	+					
RHD*DAU0 - *DAU3, *DAU8 - *DAU16	RHD*10.00-*10.03, *10.08 - *10.16	cDe	+	+	+	+	+	+	+	+	-	+					+
RHD*DAU4, *DAU5*[4,5], -*DAU6.01*[4,5]	RHD*10.04, *10.05*[4,5], *10.06.01*[4,5]	cDe	+	+	+	+	+	+	+	+	+	+					-
RHD*DBT.1	RHD*14.01	CDe	+	+	+	+					+	+					
RHD*DBT.2*[4,5]	RHD*14.02*[4,5]	CDe	+	+	+	+					+	+					
RHD*DFR1 -*DFR5, *DFR6 ◆ oder / or RHD*.U ◆	RHD*17.01 - *17.03, *17.03 oder / or RHD*69N.01		+	+	+		+	+	+	+	+	+					
RHD*DHMI	RHD*19	cDE	+	+	+	+	+	+	+	+	+	+		+			
RHD*DNB	RHD*25	CDe	+	+	+	+	+	+	+	+	+	+			+		
RHCE*ce-DIS(-ce (RH:33 (DHAR+))	RHCE*01.22	cDe					-	+									
RHD*DEL1-8(-D	RHD*01N.02	cDE										+					
RHD*D-CE(2-9)-D	RHD*01N.03	CDe	-	+								+					
RHD*D-CE(3-7)-D	RHD*01N.06	CDe oder / or (C)de*	+	+						+	+	+					
RHD*D-CE(4-7)-RHD1	RHD*01N.07	cDE	+	+	+						+	+					
RHD*D-CE(4-7)-RHD2	RHD*01N.07	cDE	+	+	+					+	+	+					
RHD*D-CE(8-9)-D		Cde	+	+	+	+	+	+	+			+					
RHD(delE+9) ◆		CDe	+	+	+	+	+	+	+	+		+					
RHD*D-CE(10) ◆		Cde	+	+	+	+	+	+	+	+	-	+					
RHD(Q41X)*[4,5]	RHD*01N.09	Cde	-	+	+	+	+	+	+	+	+	+					
DCS, [DFW, DHR, DIM, DNU] *[4,5]		diverse, various	-	-	-	-	-	-	-	-	-	-					

Genotyp / Genotype	Phänotyp / Phenotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

n.t. / [ ] n.t. = not tested currently

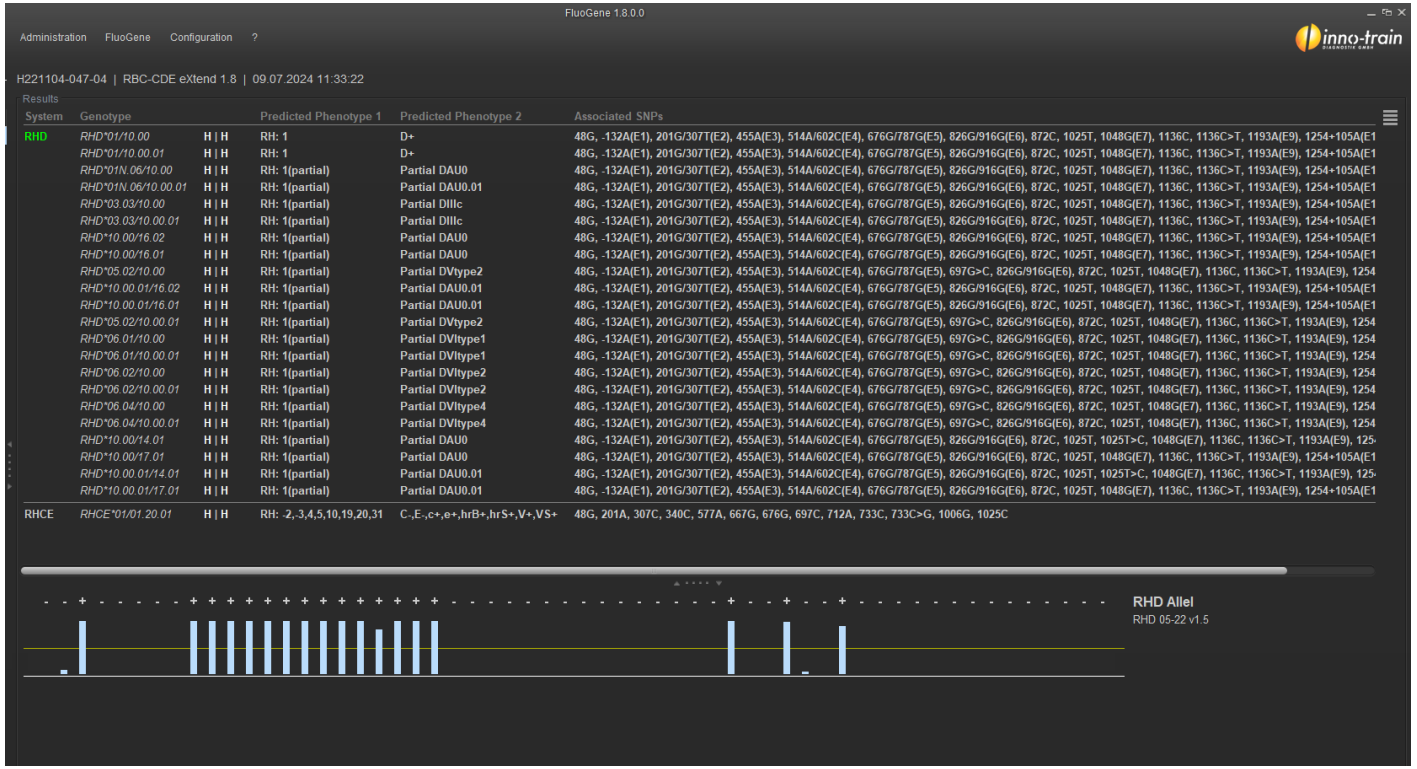
Beispiel siehe Kurzanleitung / *Please see short instructions for examples*

Proben-ID / *Sample ID:*

Name / *Name:*

Geb.-Datum / *Date of birth:*

Gelbild / *Gel picture:*



System	Genotype		Predicted Phenotype 1	Predicted Phenotype 2	Associated SNPs
RHD	RHD*01/10.00	H | H	RH: 1	D+	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254+105A(E1
	RHD*01/10.00.01	H | H	RH: 1	D+	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254+105A(E1
	RHD*01N.06/10.00	H | H	RH: 1(partial)	Partial DAU0	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254+105A(E1
	RHD*01N.06/10.00.01	H | H	RH: 1(partial)	Partial DAU0.01	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254+105A(E1
	RHD*03.03/10.00	H | H	RH: 1(partial)	Partial DIIIc	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254+105A(E1
	RHD*03.03/10.00.01	H | H	RH: 1(partial)	Partial DIIIc	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254+105A(E1
	RHD*10.00/16.02	H | H	RH: 1(partial)	Partial DAU0	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254+105A(E1
	RHD*10.00/16.01	H | H	RH: 1(partial)	Partial DAU0	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254+105A(E1
	RHD*05.02/10.00	H | H	RH: 1(partial)	Partial DVtype2	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 697G>C, 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254
	RHD*10.00.01/16.02	H | H	RH: 1(partial)	Partial DAU0.01	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254+105A(E1
	RHD*10.00.01/16.01	H | H	RH: 1(partial)	Partial DAU0.01	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254+105A(E1
	RHD*05.02/10.00.01	H | H	RH: 1(partial)	Partial DVtype2	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 697G>C, 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254
	RHD*06.01/10.00	H | H	RH: 1(partial)	Partial DVItype1	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 697G>C, 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254
	RHD*06.01/10.00.01	H | H	RH: 1(partial)	Partial DVItype1	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 697G>C, 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254
	RHD*06.02/10.00	H | H	RH: 1(partial)	Partial DVItype2	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 697G>C, 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254
	RHD*06.02/10.00.01	H | H	RH: 1(partial)	Partial DVItype2	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 697G>C, 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254
	RHD*06.04/10.00	H | H	RH: 1(partial)	Partial DVItype4	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 697G>C, 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254
	RHD*06.04/10.00.01	H | H	RH: 1(partial)	Partial DVItype4	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 697G>C, 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254
	RHD*10.00/14.01	H | H	RH: 1(partial)	Partial DAU0	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1025T>C, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 125
	RHD*10.00/17.01	H | H	RH: 1(partial)	Partial DAU0	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254+105A(E1
	RHD*10.00.01/14.01	H | H	RH: 1(partial)	Partial DAU0.01	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1025T>C, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 125
	RHD*10.00.01/17.01	H | H	RH: 1(partial)	Partial DAU0.01	48G, -132A(E1), 201G/307T(E2), 455A(E3), 514A/602C(E4), 676G/787G(E5), 826G/916G(E6), 872C, 1025T, 1048G(E7), 1136C, 1136C>T, 1193A(E9), 1254+105A(E1
RHCE	RHCE*01/01.20.01	H | H	RH: -2,-3,4,5,10,19,20,31	C-,E-,c+,e+,hrB+,hrS+,V+,VS+	48G, 201A, 307C, 340C, 577A, 667G, 676G, 697C, 712A, 733C, 733C>G, 1006G, 1025C

CDE eXtend RHD: which variants to want to discriminate?

RHD		BAG	CDE eXtend	USA TOTAAL	HILA TOTAAL	SSA	(ISBT 004) RHD blood group alleles v6.4 31-JUL-2023.xlsx	NOTE
RHD*01	DPOS	X	X	0,279	0,11	0,5448		
RHD*01W.1	WD type 1	X	X	0,17	0,42	0,0008	**c.809T>G**	
RHD*01W.2	WD type 2	X	X	0,114	0,2371		**c.1154G>C**	
RHD*01W.1.1	WD type 1.1	X	X	0,0000	0,24		c.52C>G **c.809T>G**	
RHD*01N.01	DNEG	X	X	0,0000	0,05	0,1455	**RHD deletion**	
RHD*10.00	DAU0	X	X	0,002	0,02	0,1507	**c.1136C>T**	
RHD*09.03.01	WD type 4.0	X	X	0,099	0,032	0,0212	**c.602C>G** c.667T>G c.819G>A	1
RHD*09.01.00	DAR1.00 (WD 4.2)	X	X	0,081	0,0415	0,0029	**c.602C>G** c.667T>G **c.1025T>C**	
RHD*01W.3	WD type 3	X	X	0,034	0,034		**c.8C>G**	
RHD*08N.01	Psi	X	X	0,006	0,005	0,0344	**c.-7061del exon 1** to 10 c.609G>A c.654G>C c.667T>G c.674C>T c.807T>G	
RHD*03.01	DIIIa		X	0,019	0,0008	0,0104	**c.186G>T c.410C>T** c.455A>C **c.602C>G** c.667T>G c.819G>A	
RHD*03N.01	DIIIa-CEVS (4-7)-D		X	0,0000	0,0000	0,0286	**c.186G>T c.410C>T** c.455A>C **CE exons 4-7 with 733C>G 1006G>T**	
RHD*04.01	DIVa	X	X	0,014	0,0004	0,0124	**c.186G>T c.410C>T** c.455A>C **c.1048G>C**	
RHD*06.01	DVI.1	X	X	0,001	0,02		c.505A>C c.509T>G c.514A>T c.544T>A c.577G>A c.594A>T **c.602C>G** c.667T>G c.676G>C c.697G>C c.712G>A c.733G>C c.744C>T c.787G>A c.800A>T	1, 2
RHD*10.03	DAU3	X	X	0,0000	0,0000	0,0205	**c.835G>A c.1136C>T**	
RHD*06.02	DVI.2	X	X	0,0000	0,018		c.505A>C c.509T>G c.514A>T c.544T>A c.577G>A c.594A>T **c.602C>G** c.667T>G c.697G>C c.712G>A c.733G>C c.744C>T c.787G>A c.800A>T c.916G>A c.932A>G	1,2
RHD*10.05	DAU5	X	X	0,005	0,0000	0,0092	c.667T>G **c.697G>C c.1136C>T**	
RHD*10.00.01	DAU0.01	X	X	0,0000	0,0000	0,0119	c.579G>A **c.1136C>T**	
RHCE*01.22	RHCE*CE-D(5)-CE	X	X	0,0000	0,01		**Alles behalve E5 = CE**	
RHD*12.01	DOL			0,005	0,0000	0,0023	c.509T>C c.667T>G	3
RHD*01W.5	WD type 5	X	X	0,0000	0,005		**c.446C>A**	
RHD*25	DNB	X	X	0,001	0,0034		**c.1063G>A**	
RHD*10.04	DAU4	A	X	0,001	0,0000	0,0034	**c.697G>A c.1136C>T**	
RHD*15	WP type 15	X	X	0,0000	0,0041		**c.845G>A**	
RHD*06.03	DVI.3	X	X	0,0000	0,004		c.361T>A c.380T>C c.383A>G c.455A>C c.505A>C c.509T>G c.514A>T c.544T>A c.577G>A c.594A>T **c.602C>G** c.667T>G c.697G>C c.712G>A c.733G>C c.744C>T c.787G>A c.800A>T	1, 2

ISBT tabel : [004 RHD Alleles | The International Society of Blood Transfusion (ISBT) (isbtweb.org)]

Flemish population:

Mapped presence and frequency of RHD alleles in the Flemish population (Van Sandt *et al.* 2023).

Compared our population with bigger studies in US (Keller et al, 2020, Chou et al 2018, Chang et al. 2020).

RHD variants with a frequency ≥ 0,001

Relevant SNPs

Mutant en wild type SNP: zygosity of variants.

CDE eXtend

CDE eXtend RHD, which variants to want to discriminate?

Evaluation / comparison of the RHD pakket of

BAG

RHD allel	Fenotype	Relevant in testpakket	Rationale
RHD*01W.20	WD type 20	NEE	geen frequentie gevonden
RHD*02	DII	?	1 malig gevonden in HILA
RHD*01N.09	RHD*(Q41X)	?	1 malig gevonden in HILA
RHD*17:01-05	DFR	?	0,003 in publicatie Keller

Quick and easy screen of weak D type 1, 2, 3 variants
Weak D samples Caucasians

IVDR?!
Alternative?

vs

CDE eXtend

RHD allel	Fenotype	Relevant in testpakket	Rationale
RHD*01.01	D48C	JA	betere inschatting RHCE, shared SNP
RHD*W.14	WD type 14	NEE	geen frequency gevonden
RHD*01W.40	WD type 40	JA	komt voor in SCD pop
RHD*01W.51	WD type 51	NEE	geen frequency gevonden
RHD*01W.66	WD type 66	JA	komt voor in SCD pop
RHD*03.01	DIIIa	JA	rel hoge in SCD pop
RHD*03N.01	DIIIa-CEVS (4-7)-D	JA	rel hoge in SCD pop

RHD*03.01 and 03N.01, high frequency in SSA people

RHD*01 (RhD POS) does not mask the presence of underlying variants

Inclusion of WT and mutant SNPs: zygosity.

Resolving more complex cases, anti-D, patients SSA origin

Next Generation Sequening

Is NGS sequencing suitable for red blood cell genotyping?

1) Is NGS able to correctly determine the RBC alleles?
2) Is the bioinformatics tool correct in predicting geno-, feno- and haplotypes?

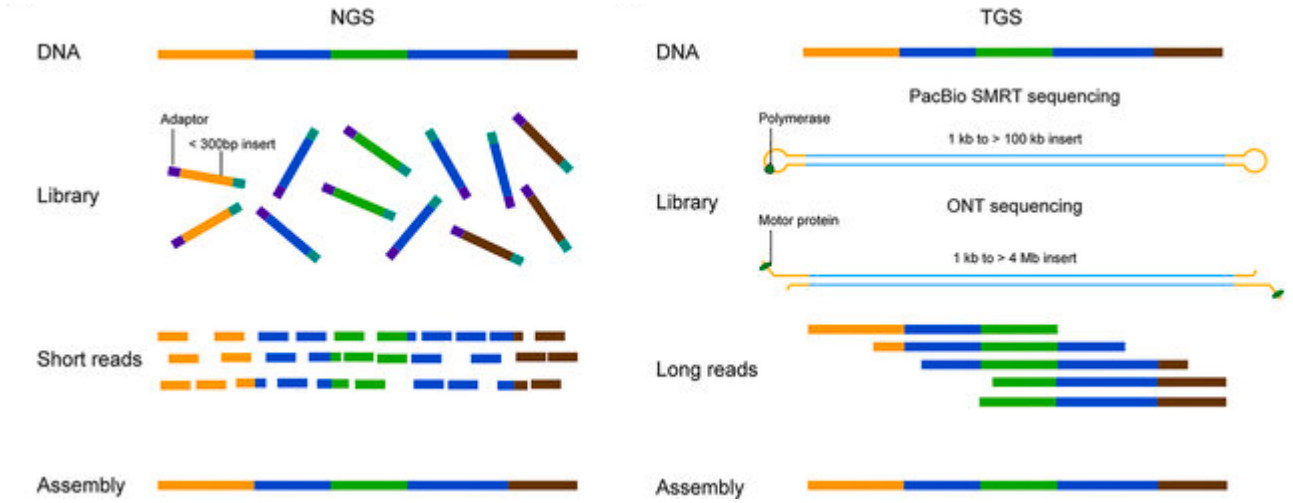
Next Generation Sequening

Which NGS strategy to use:

- Whole genome sequencing (WGS)
- Whole exome sequencing (WES)
- Target sequencing

→ Differ in complexity, limitations, cost

- Short read sequencing
- Long read sequencing



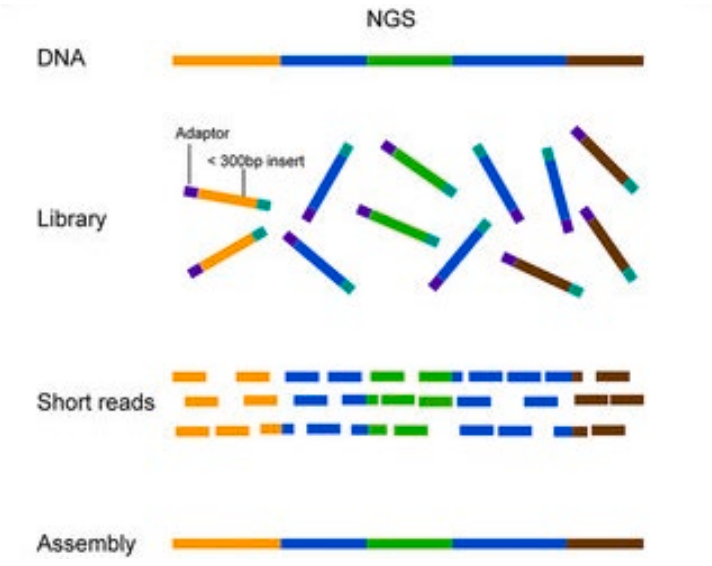
Evaluation of RBC-NGStype CORE

	RBC-NGS*type*® CORE	RBC-NGS*type*® enCORE	RBC-NGS*type*® enCORE+
Article No.:	001 110 024	001 120 024	001 130 024
Multiplexes	4	2	2
Available	Yes	Yes	Coming soon
Blood Group Systems	ABO	Lutheran	P1PK
	Rh	Yt	Diego
	MNS	Dombrock	Scianna
	Kell	Colton	Chido/Rodgers
	Kidd	Landsteiner-Wiener	Gerbich
	Duffy	Knops	Cromer
			Indian
			John Milton Hagen
	All exons and relevant non-coding region amplified		Rh-asso. glycoprotein
	Key exons and relevant non-coding region amplified		JR
			LAN

= Target sequencing

all relevant exons and non-coding regions

Short read sequening



Evaluation of RBC-NGStype CORE

Random cohort patients / donors Red Cross Flanders

~= 85 % Caucasian

k-, Kpb- …

Cw+, Cx+, Moa+, …

Classic RHCE

RHD weak D types 1, 2, 3 or Partial D VI

Evalu[illegible]on of RBC-NGStype CORE

Random cohort patie[illegible]lonors Red Cross Flanders

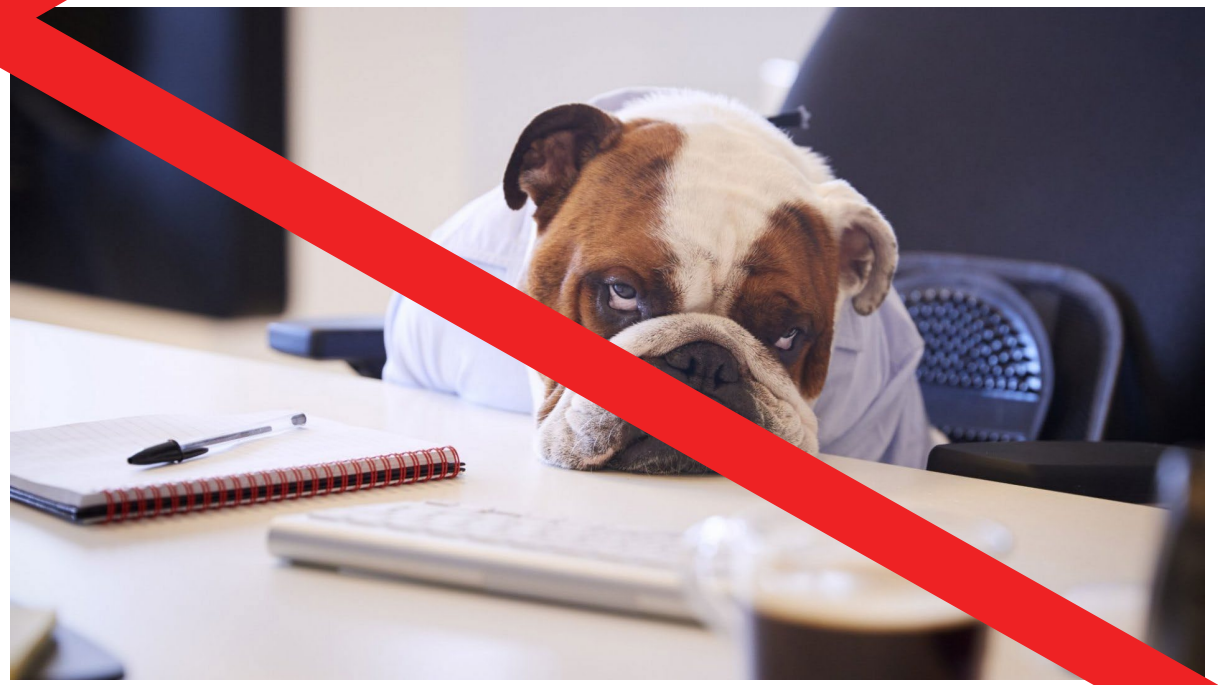
~= 85 % Caucasian

k-, Kpb- …

Cw+, Cx+, Moa+, …

Classic RHCE

RHD weak D types 1, 2, 3 or Partial D VI



Evaluation of RBC-NGStype CORE

'Selected' cohort n= 24

AIM = to challenge the kit / inno-train

Focus on the more complex systems: RHD and RHCE

Samples included:

1) Pretyped RHD – RHCE hybrid alleles
   → how does the method handle hybrid reads/exons?

2) Cases where standard SNP testing not result in a genotype
   → Is NGS able to give more information / find rare/ new alleles?

3) FYGATA positive samples

Prevalence of RH variants

Rode Kruis Vlaanderen

PROXY = FYGATA mutation

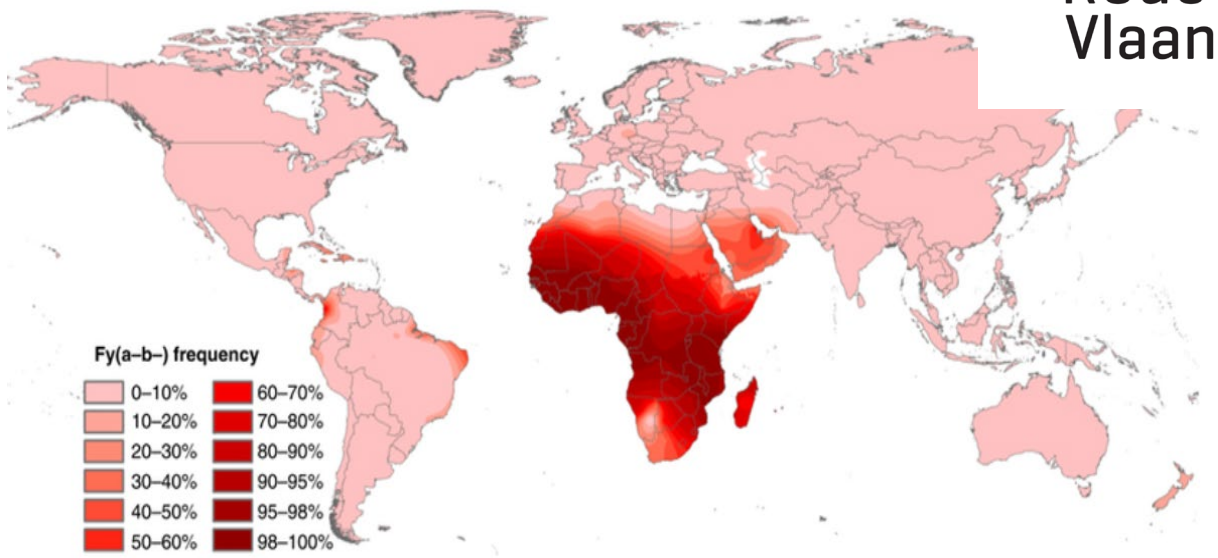
Populatie Sub Sahara Africans

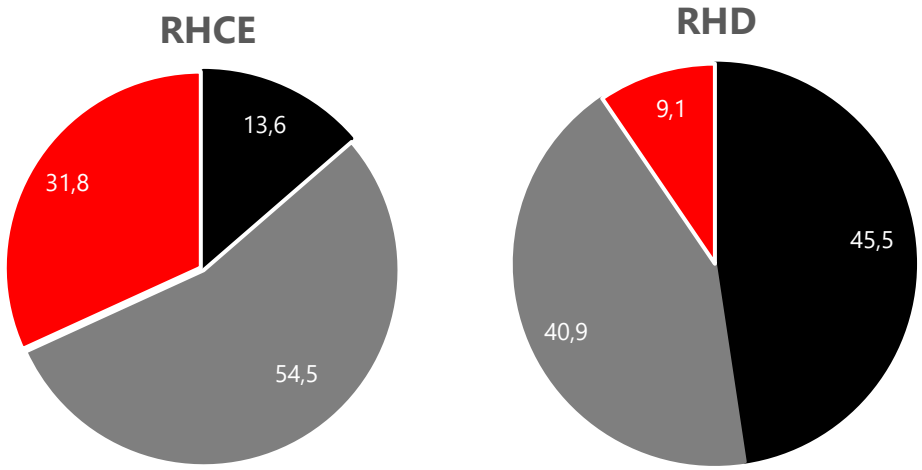
n =22

No variant

1 variant

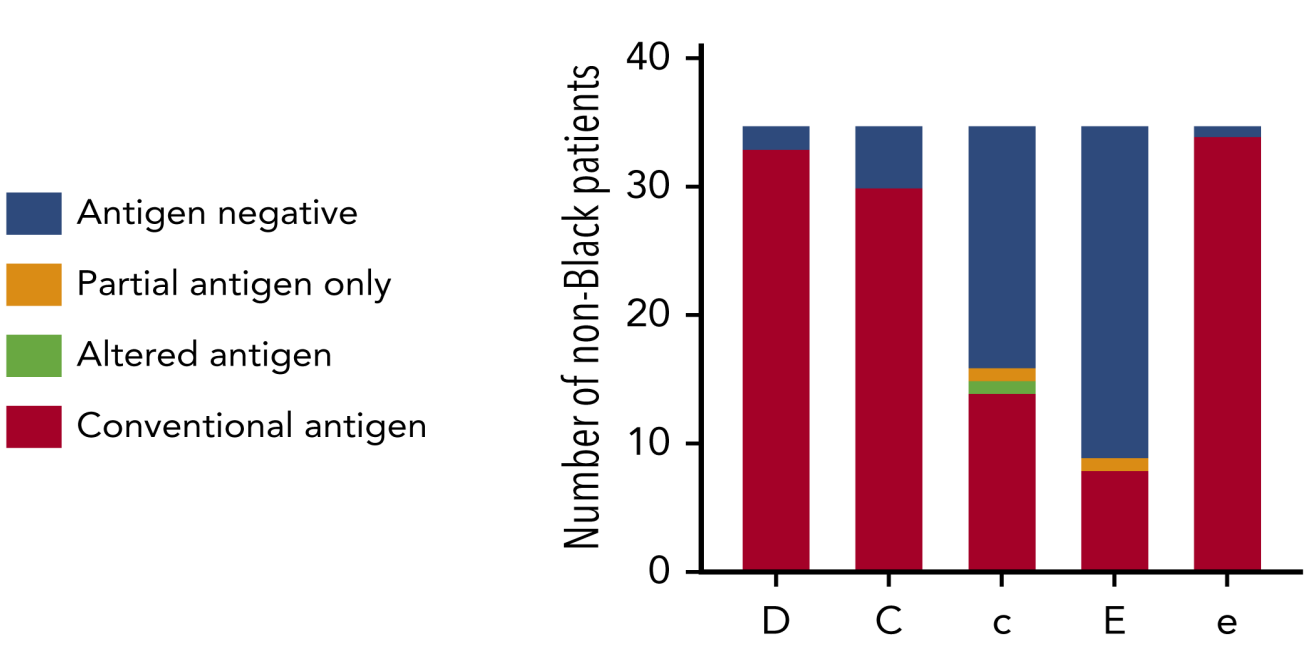
2 variants (compound heterozygous)

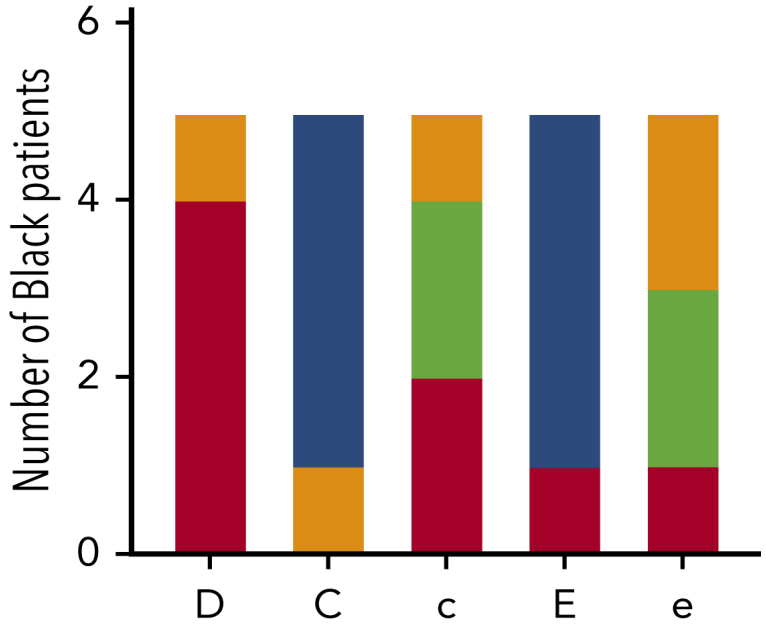




n= 22	RHCE variant	RHD variant
RHCE variant	19	11 (58%)
RHD variant	11 (100%)	11

Prevalence of RH variants





Waldis et al. 2021

Origine Sub-Sahara Africa hints for the presence of a RH variant.

Evaluation of RBC-NGStype CORE

'Selected' cohort n= 24

AIM = to challenge the kit / inno-train

Focus on the more complex systems: RHD and RHCE

Samples included:

1) RHD – RHCE hybrid alleles
   → how does the method handle hybrid reads/exons?

2) complex cases where standard SNP testing not result in a genotype
   → Is NGS able to give more information / find rare/ new alleles?

3) FYGATA positive samples
   → **Is NGS able to resolve combinations of RHCE and RHD variants?**


On the road again!
inno-train
DIAGNOSTIK GMBH


CHALLENGE
ACCEPTED

Short read sequencing

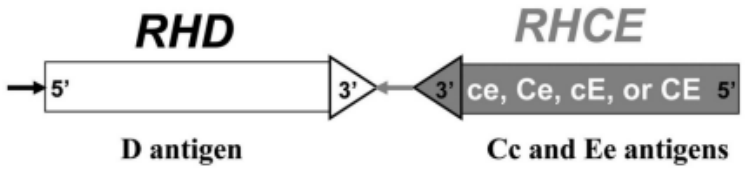
RBC NGS Type = exon amplification approach

Sample

Amplify and sequence the exons

Bioinformatics: sort them per locus

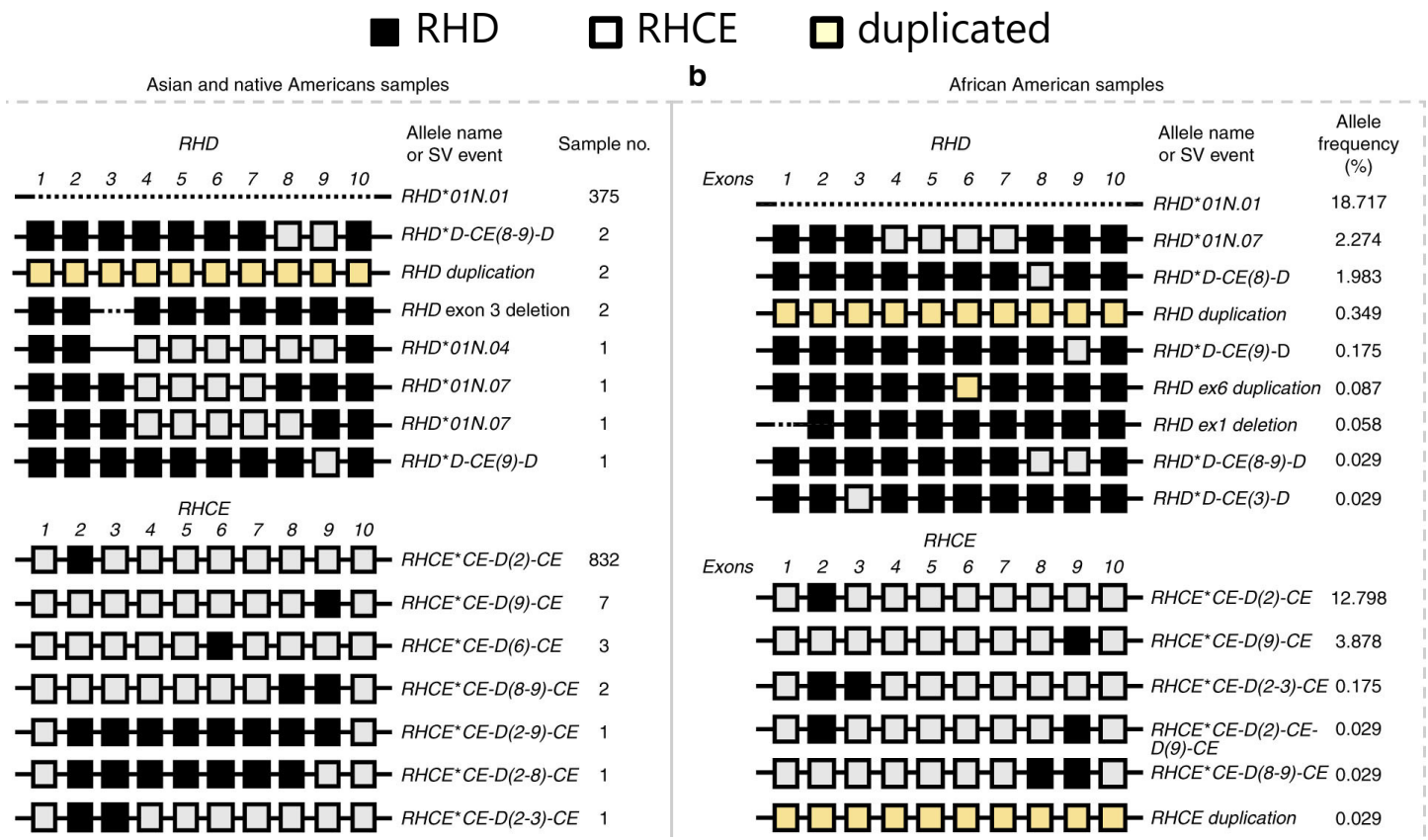
1) RHD – RHCE hybrid alleles



gDNA = 97% identical

In hybrids complete exons are exchanged between RHD and RHCE:

How does NGS bioinformatics sort the short reads correct when exons of RHD and RHCE are identical?



Wheeler *et al* 2019, Genomic characterization of the *RH* locus detects complex and novel structural variation in multi-ethnic cohorts

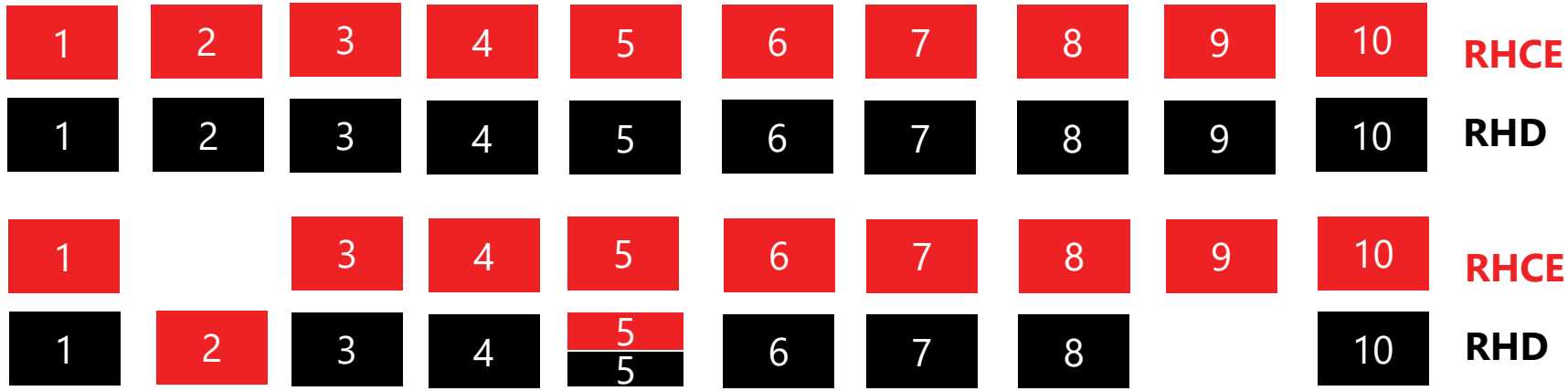
Short read sequencing

RBC NGS Type = exon amplification approach

Sample

Amplify and sequence the exons

Bioinformatics: sort them per locus



1) RHD – RHCE hybrid alleles

SAMPLE 1 L220330-155

Reference results:

SEROLOGY	RhD NEG		
BAG WEAK D TYPE	No WEAK D		
BAG PARTIAL D TYPE	RHD*01N.05	EXONS 3-7 CE	→ phenotype D-
CDE eXtend	RHD*03N.01	EXONS 4-7 CE + SNPs	→ phenotype D- C+

1) RHD – RHCE hybrid alleles

SAMPLE 1

Reference results:

SEROLOGY	**RhD NEG**		
BAG WEAK D TYPE	**No WEAK D**		
BAG PARTIAL D TYPE	**RHD*01N.05**	**EXONS 3-7 CE**	**→ phenotype D-**
CDE eXtend	**RHD*03N.01**	**EXONS 4-7 CE + SNPs**	**→ phenotype D-** C+

1) RHD – RHCE hybrid alleles

SAMPLE 1

Reference results:

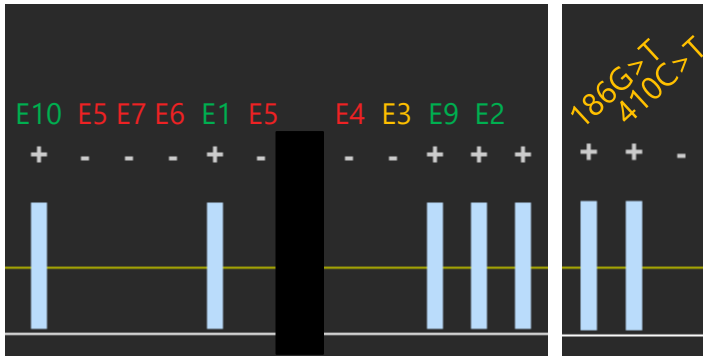
SEROLOGY RhD NEG

BAG WEAK D TYPE No WEAK D

BAG PARTIAL D TYPE RHD*01N.05 EXONS 3-7 CE → phenotype D-

RHD*01	RHD pos.		+	+	+	+	+	+	+	+	+	+
RHD*D-CE(3-7)-D	RHD*01N.05	CDe oder / or (C)de^5	+	+						+	+	+

CDE eXtend RHD*03N.01/ RHD*01N.01 EXONS 4-7 CE + SNPs E2 E3 → phenotype D- C+



CDE eXtend is probably most correct answer

1) RHD – RHCE hybrid alleles

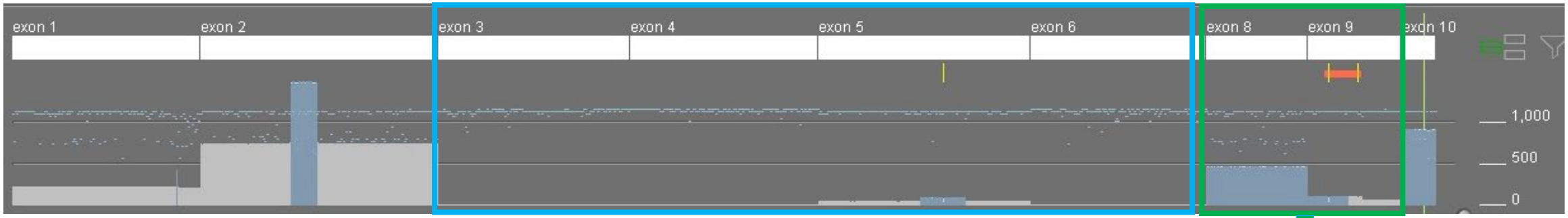
SAMPLE 1

RHD result:

CDE eXtend	RHD*03N.01	CE exons 4-7
RBC NGS type	**RHD*03N.02**	**CE exons 4-9**

Very low coverage exons 3-6

Exon 7 data missing: RHCE



Exon 8-9

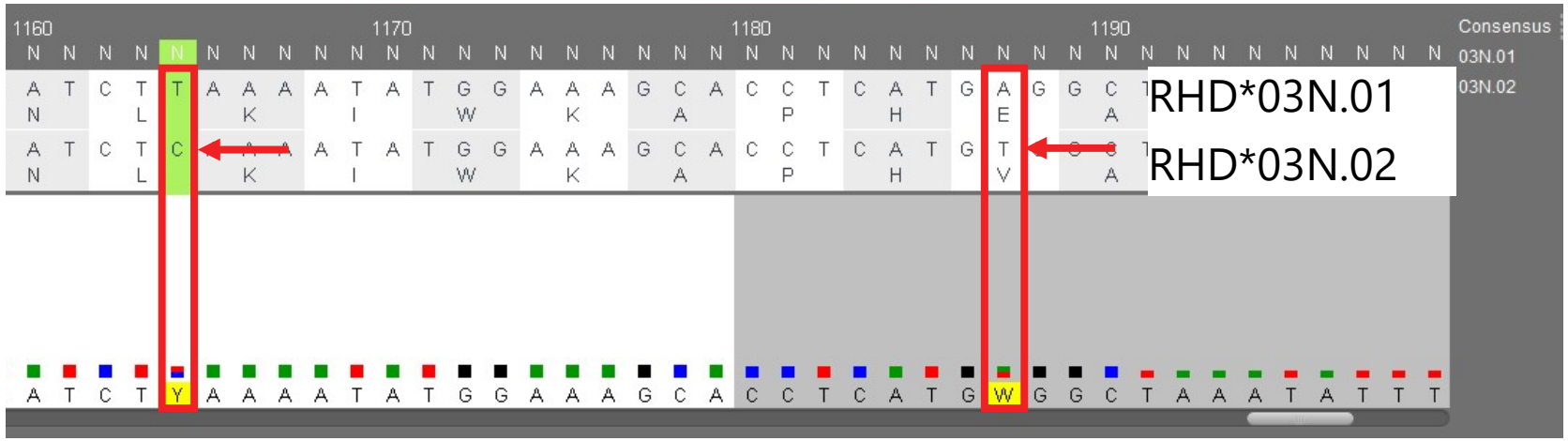
RHCE or RHD?

1) RHD – RHCE hybrid alleles

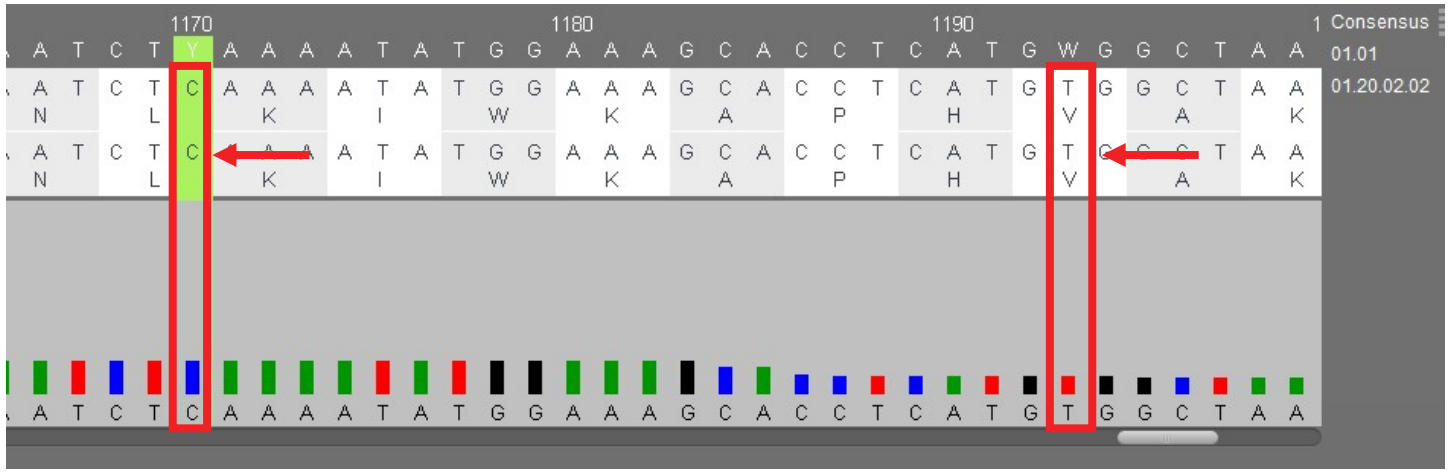
SAMPLE 1

CDE eXtend	RHD*03N.01	CE exons 4-7
RBC NGS type	**RHD*03N.02**	**CE exons 4-9**

RHD



RHCE



Crosstalk D-CE ?

Looks like CE

BUT

Heterozygous SNPs +/- in balance

What is the correct genotype?
SSP vs NGS?

Homology between RHD and RHCE can cause crosstalk and mistyping

1) RHD – RHCE hybrid alleles

SAMPLE 2 H221004-077

Reference results:

SEROLOGY	RhD NEG
BAG WEAK D TYPE	Weak D type 20
BAG PARTIAL D TYPE	RHD(1-9)-CE(10)
CDE eXtend	RHD*01EL.11

EXON 10	c.1250T>C
EXON 10 CE	
E10	c1252-1253 ins T

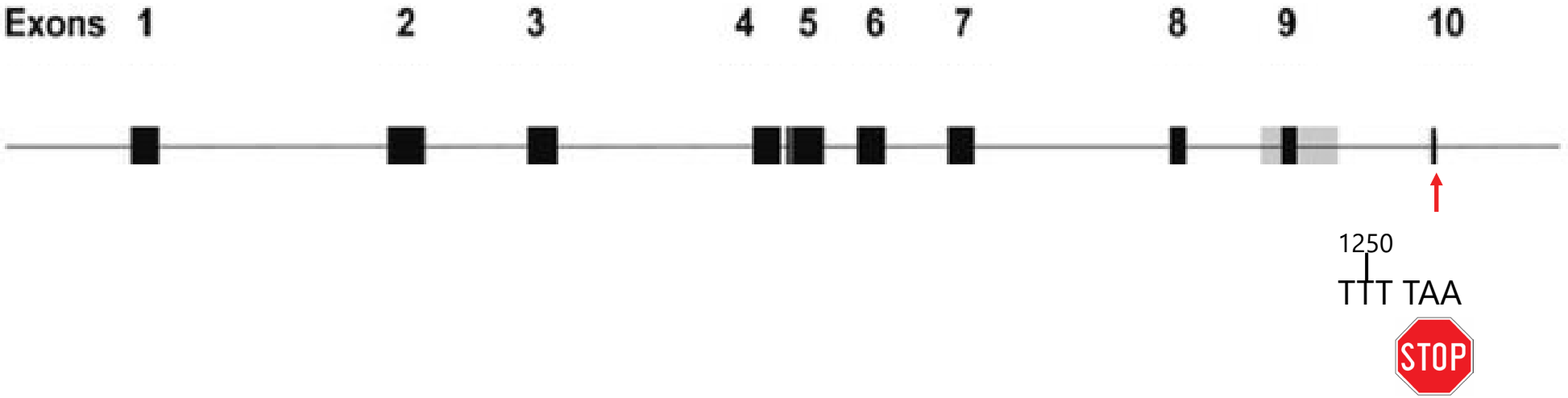
1) RHD – RHCE hybrid alleles

SAMPLE 2

Reference results:

SEROLOGY	RhD NEG
BAG WEAK D TYPE	Weak D type 20
BAG PARTIAL D TYPE	RHD(1-9)-CE(10)
CDE eXtend	RHD*01EL.11

EXON 10	c.1250T>C
EXON 10 CE	
E10	c1252-1253 ins T

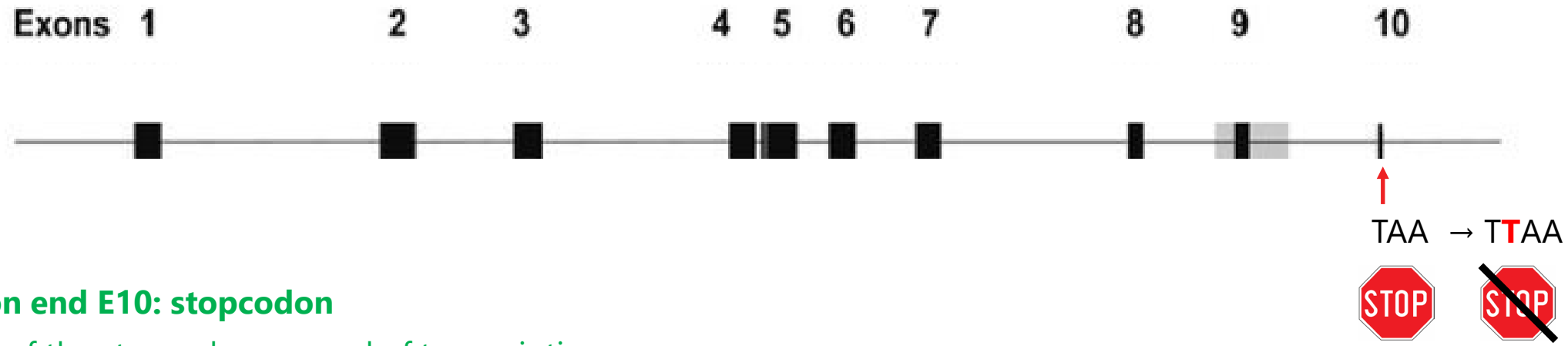


1) RHD – RHCE hybrid alleles

SAMPLE 2

Reference results:

SEROLOGY	**RhD NEG**		
~~BAG WEAK D TYPE~~	~~Weak D type 20~~	~~EXON 10~~	~~c.1250T>C~~
~~BAG PARTIAL D TYPE~~	~~RHD(1-9)-CE(10)~~	~~EXON 10 CE~~	
CDE eXtend	**RHD*01EL.11**	**E10**	**c1252-1253 ins T**



Alteration end E10: stopcodon

Mutation of the stopcodon: no end of transcription

1) RHD – RHCE hybrid alleles

SAMPLE 2

BAG results incorrect?

RHD*01EL.11 : alters primerbinding sites for BAG primers?

BAG WEAK D TYPE Weak D type 20 EXON 10 c.1250T>C

Aspecific binding of primer?

BAG PARTIAL D TYPE RHD(1-9)-CE(10) EXON 10 CE

Failure of the specific amp exon 10 results in RHD(1-9)-CE(10)

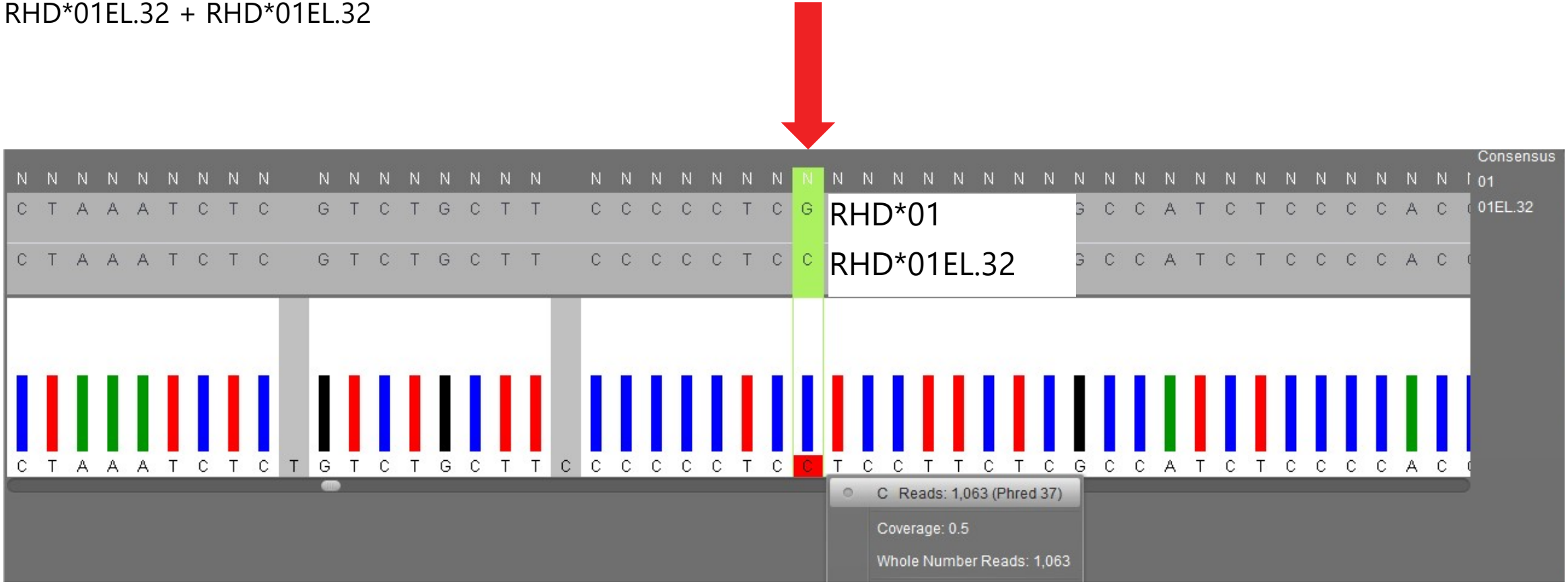
	1	2	3	4	5	6	7	8	9	10	11	12 ◆	13	14	15
	134	146	118	135	132	132	120	673	184	132	166	107	117	129	140
RHD Exons * Mix 8: Intron 7/ Exon 8	D_1	D_2	D_3	D_4	D_5	D_6	D_7	$D_{7/8}$*	D_9	D_{10}	D_2	D_6	D_7	D_7	D_8
RHD*D-CE(10) ◆	+	+	+	+	+	+	+	+	+						

1) RHD – RHCE hybrid alleles

SAMPLE 2

RBC NGStype

RHD*01EL.32 + RHD*01EL.32

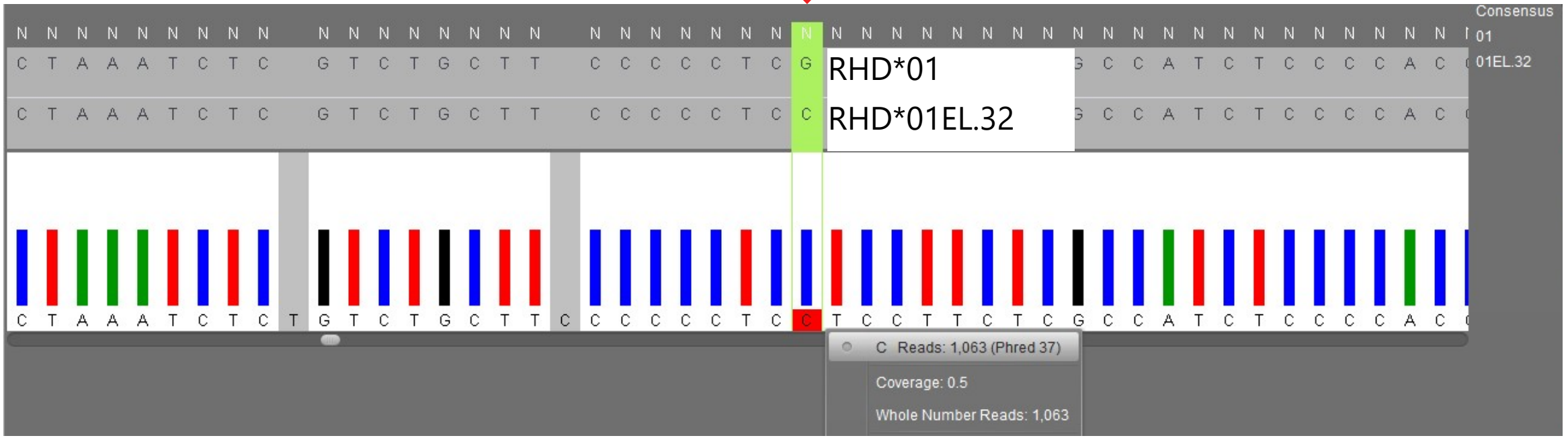


1) RHD – RHCE hybrid alleles

SAMPLE 2 H221004-077

RBC NGStype

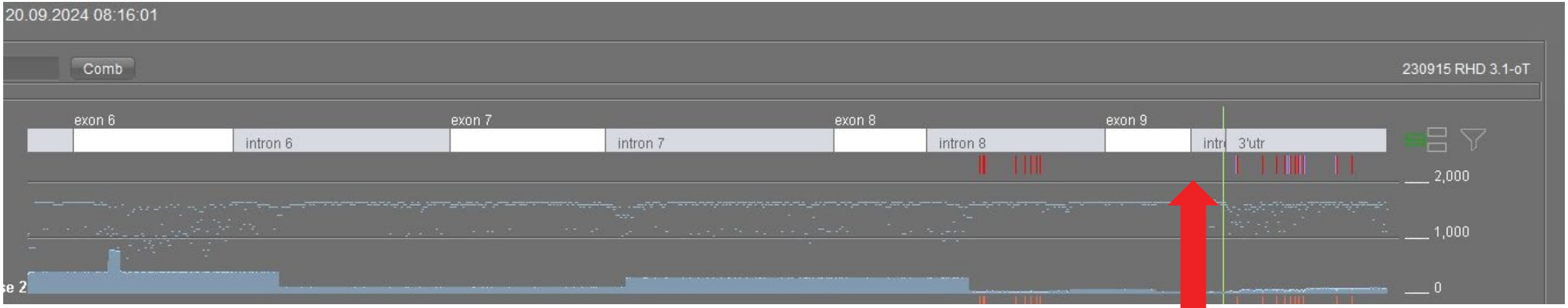
RHD*01EL.32 + RHD*01EL.32 matches serology D NEG



1) RHD – RHCE hybrid alleles

SAMPLE 2 H221004-077

RBC NGStype	**RHD*01EL.32 + RHD*01EL.32**	**E10?**	
CDE eXtend	**RHD*01EL.11**	**E10**	**c1252-1253 ins T**



NGS result incomplete:

Should be flagged: missing data, implies ambiguïties!

Check primerbinding site exon 10

NO EXON 10

Failure amplification?

Result is calculated without EXON 10 data

CDE eXtend + NGS CORE give most correct & complete answer.

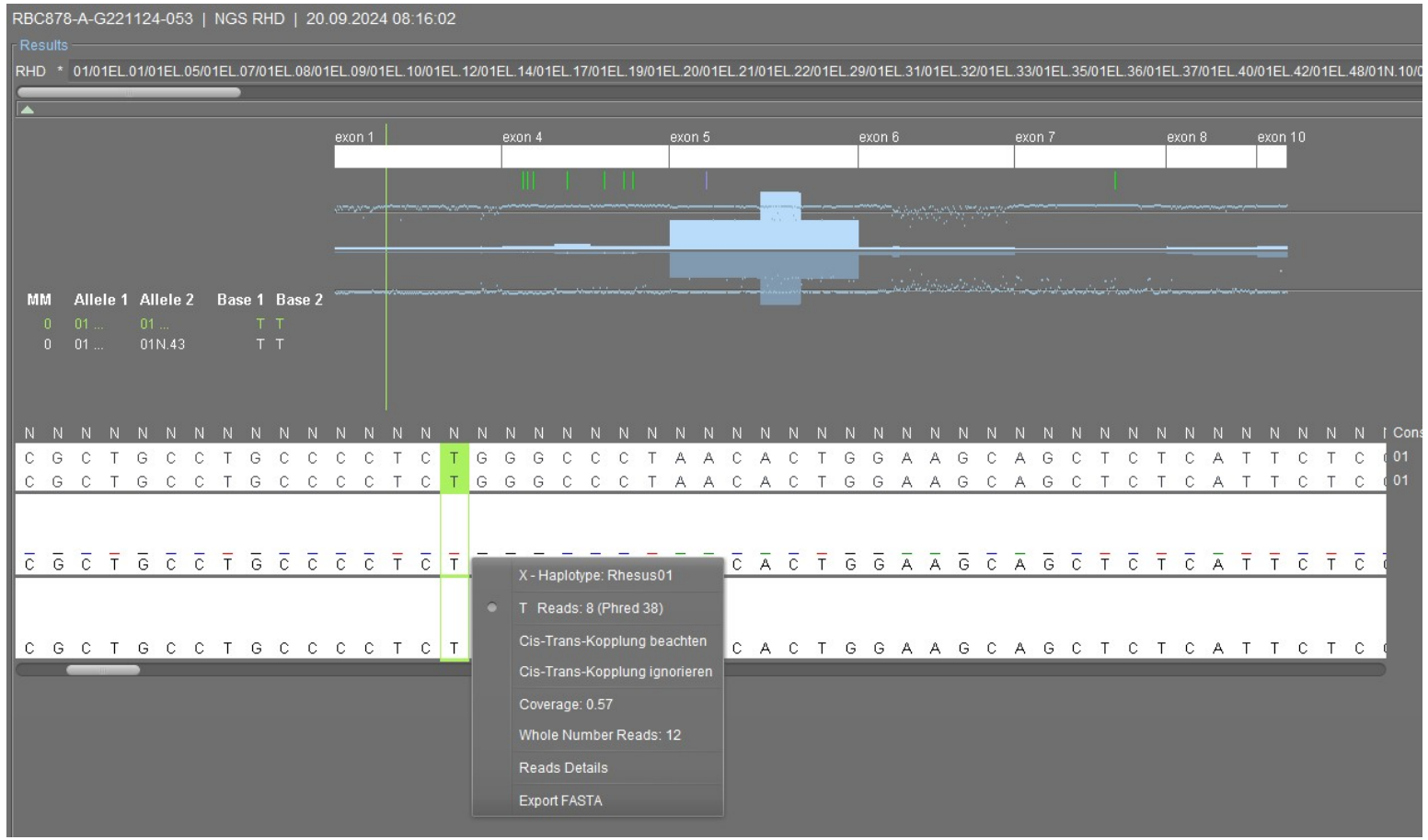
What is the correct genotype?
SSP vs NGS?

NGS allows detection of rare/new SNPs

RHD-CE hybrid alleles:
Absence of exons should be taken into account in result calculation:
Is it a RHD-CE hybrid?
Is it an exon amplification failure?

1) RHD – RHCE hybrid alleles

SAMPLE 3 G221224-053



- Exons missing
- Very low coverage of all exons except exon 5 (+200)

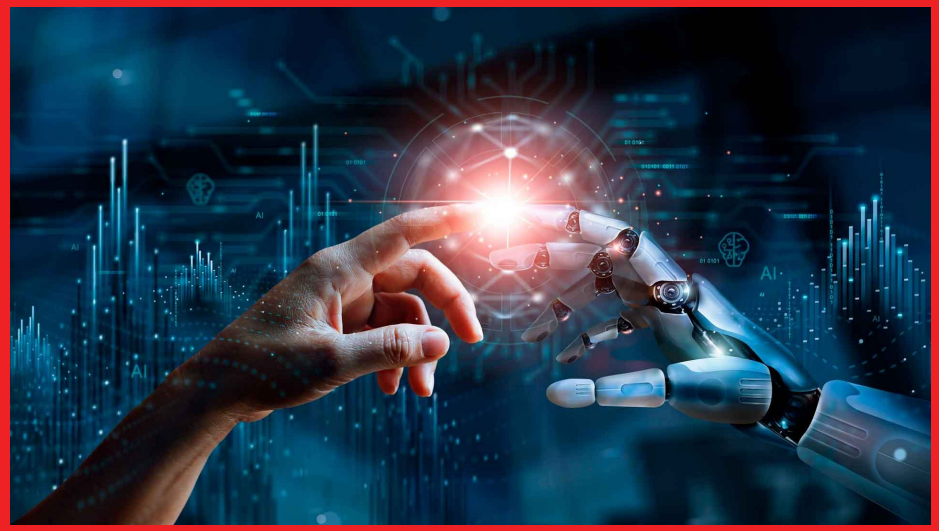
Is what we see a low coverage example of RH*01?

Is what we see pure cross talk? RHD*01N.01?

Reference data: **RHCE-D(5)-CE**

RHD-CE hybrids

Sorting the reads to the correct locus is difficult when exons are the same for RHD and RHCE.



OI/AI:

check of exon - allele - locus balance

What is the origin, expected haplotype?

2) Cases where standard SNP testing doesn't result in a explanatory genotype

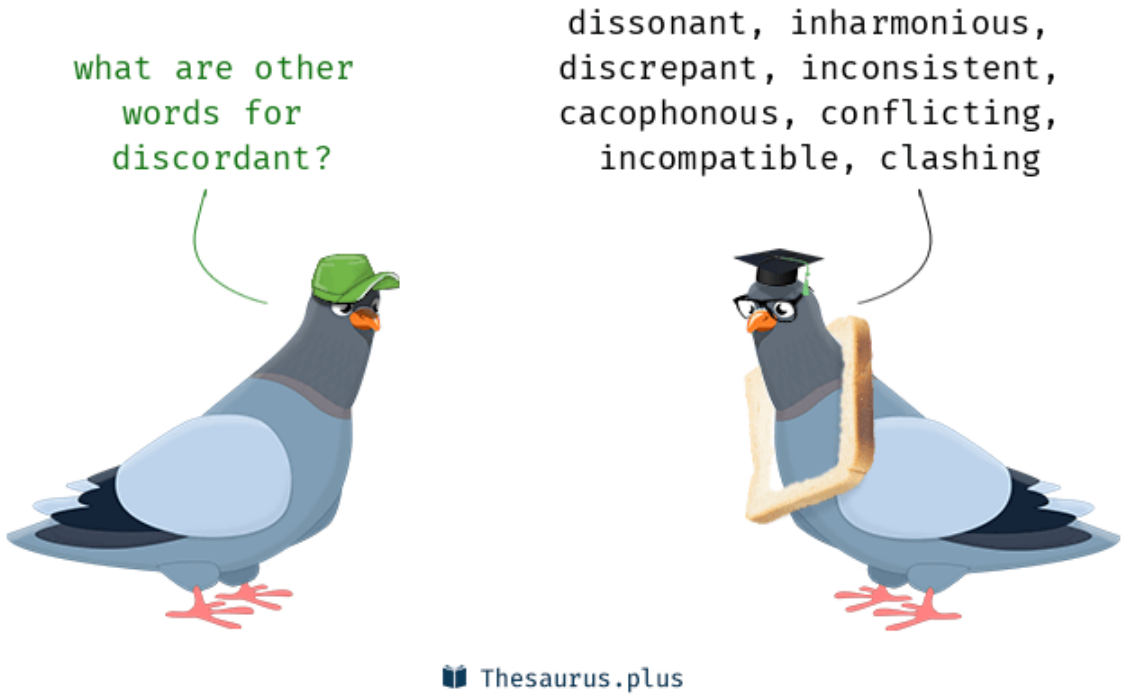
SAMPLE 4

Reference results:

SEROLOGY	**0,5+**
BAG WEAK D TYPE	No WEAK D
BAG PARTIAL D TYPE	RHD*01
CDE eXtend	RHD*01/RHD*01N.01 + amb.

NO VARIANT?

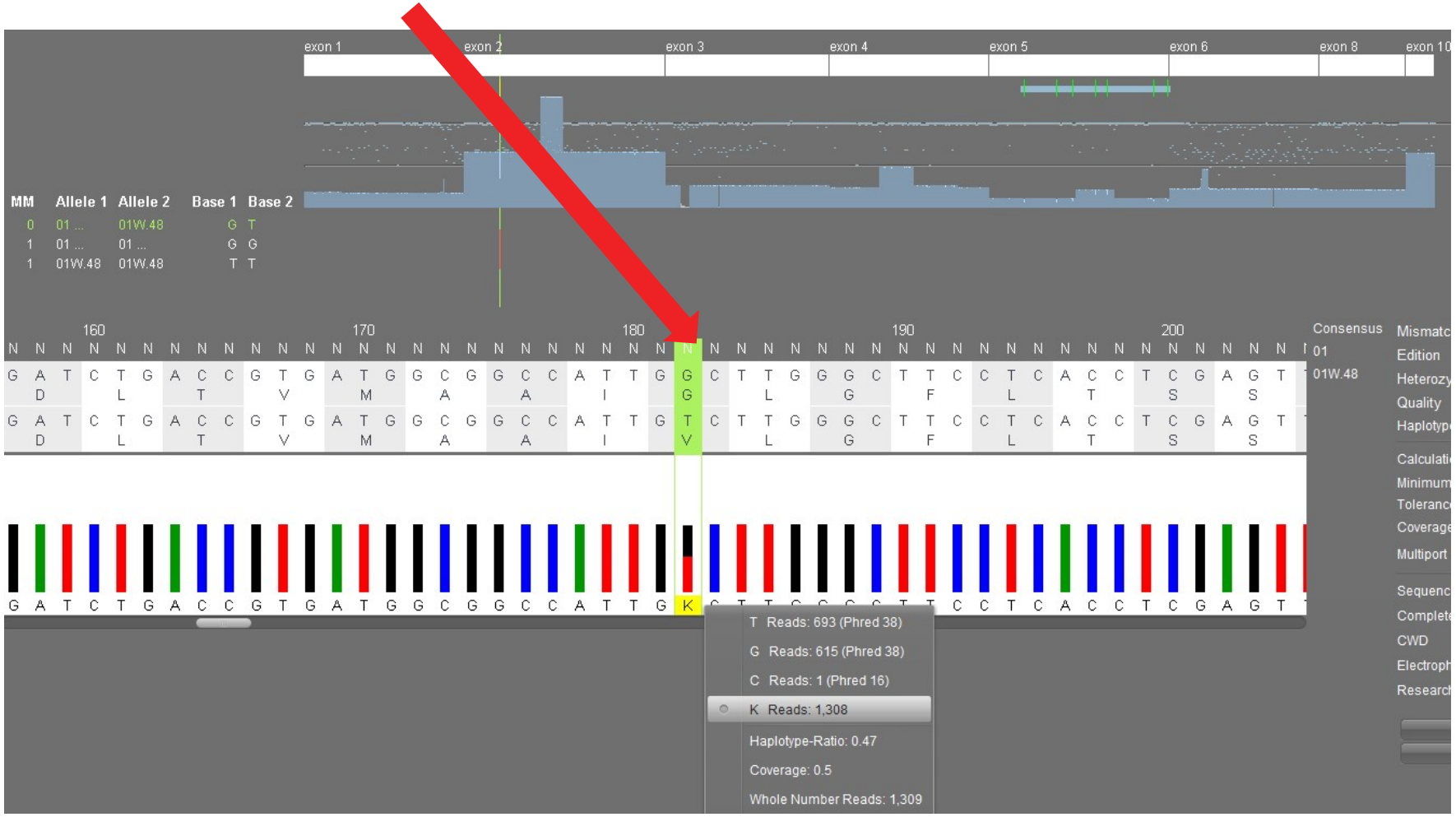
TEST LIMITATION SSP

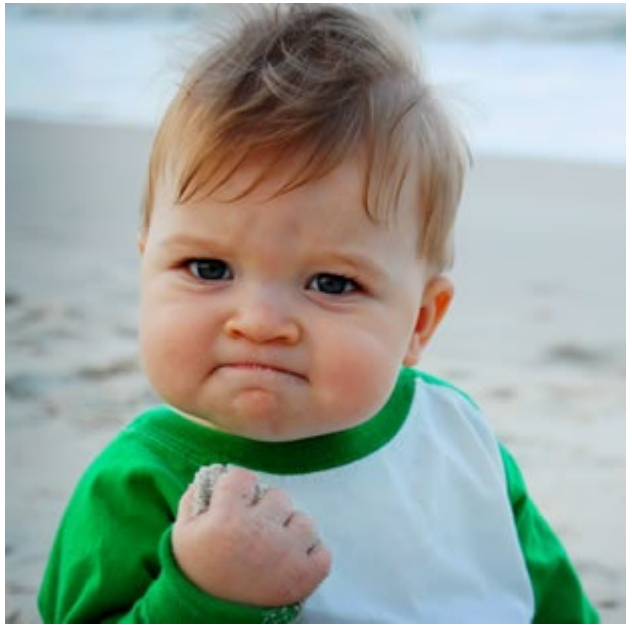


2) Cases where standard SNP testing doesn't result in a explanatory genotype

SAMPLE 4

RBC NGS CORE: **RHD*01W.48** + RHD*01.EL32





2) Cases where standard SNP testing doesn't result in a explanatory genotype

SAMPLE 4

RBC NGS CORE: **RHD*01W.48** + RHD*01.EL32

SEROLOGY **0,5+**

RHCE: Ccee

RHESUSBASE :

weak D type 48

Structure

RHD(G61V)

Key changes from standard allele

182G>T (G61V)

ISBT allele designation

RHD*01W.48
RHD*weak D type 48

Phenotype characterization and grouping

weak D type; weakened D expression: D antigen =density of about 400 antigen/RBC

Haplotype (typical)

cDE

2) Cases where standard SNP testing doesn't result in a explanatory genotype

SAMPLE 4

RBC NGS CORE: RHD*01W.48 + **RHD*01.EL32**

SEROLOGY **0,5+**

RHCE: Ccee

RHESUSBASE :

RHD(IVS1-29G>C)

Structure

RHD(IVS1-29G>C)

Key changes from standard allele

IVS1-29G>C

ISBT allele designation

RHD*01EL.32
RHD*DEL32

Haplotype (typical)

CDe

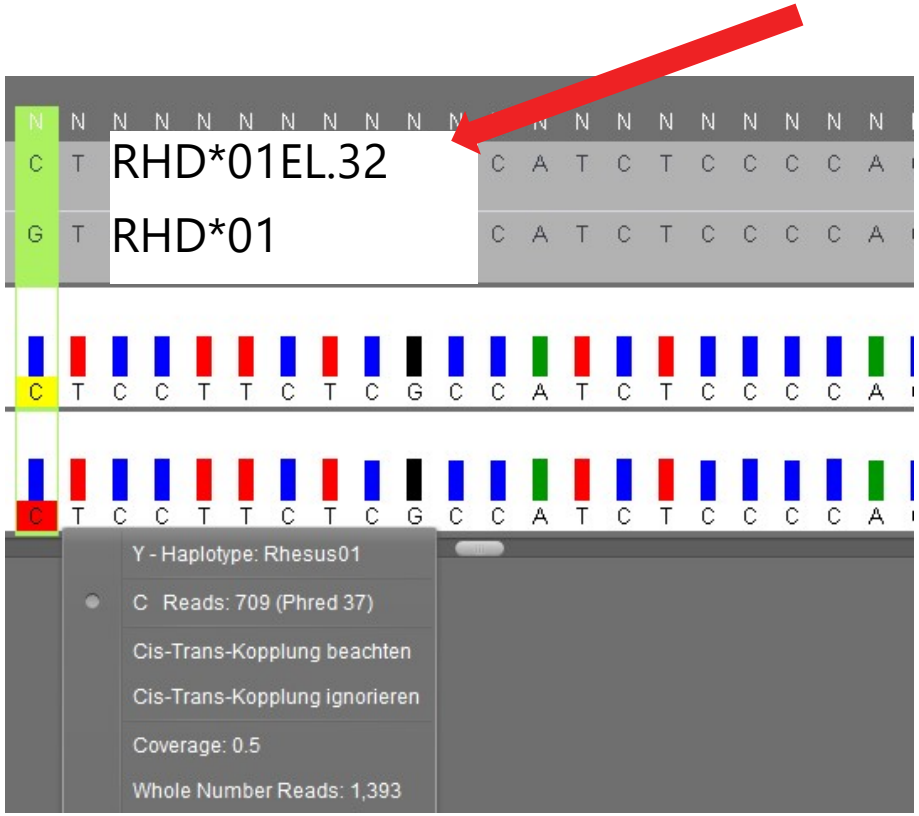
Additional comments

The relation of the IVS1-29G>C mutation to the phenotype is unsure, because IVS1-29G>C is a frequent polymorphism.

2) Cases where standard SNP testing doesn't result in a explanatory genotype

SAMPLE 4

RBC NGS CORE: RHD*01W.48 + RHD*01.EL32



In our cohort 2 samples:
RHD*01EL.32 + RHD*01EL.32 **SEROLOGY: 4+ !!!**

Is this result correct?

What is the correct phasing of both SNPs?

Is there another SNP present explaining the weak RHD phenotype?
Outside target region? Promotor?

Short read NGS can

contribute in elucidating unknown SNP to phenotype relations

Complete / Check/ Correct reference c or gDNA data

limited to the target of amplification

3) Samples with RHCE and RHD variants

SAMPLE 5 H230614-124 FYGATA

		RHD	RHCE
SEROLOGY		RhD POS	ccee
CDE eXtend	**HF**	**RHD*05.01/04/08 + 09.01**	**RHCE*01.04.01 + RHCE*01.20.01**
NGS	**0MM**	RHD* 01(/01W.150/01W.41) +09.02.01 OR **RHD*05.04 + 09.01.02**	**RHCE*01.04.01 + RHCE*01.20.01**/ OR RHCE01.04.04 + RHCE*01.20.02

SSP and NGS agree !

CDE eXtend gives a good prediction,

NGS checks if other SNPs were missed using SSP.

SSP and exon based NGS lack exon/ SNP phasing: results can be ambigues.

Prediction of the most likely result based on Origin ~ Frequency

Sub-Sahara African: homozygous FYGATA , UVAR

RHD* 01(/01W.150/01W.41) +09.02.01

OR

RHD*05.04 + 09.01.02

RHD		SSA	USA TOTAAL	HILA TOTAAL
RHD*01	DPOS	0,5448	0,2790	0,1100
RHD*10.00	DAU0	0,1507	0,0020	0,0200
RHD*01N.01	DNEG	0,1455	0,0000	0,0500
RHD*08N.01	Psi	0,0344	0,0060	0,0050
RHD*03N.01	DIIIa-CEVS (4-7)-D	0,0286	0,0000	0,0000
RHD*09.03.1	WD type 4.0	0,0212	0,0990	0,0320
RHD*10.03	DAU3	0,0205	0,0000	0,0000
RHD*04.01	DIVa	0,0124	0,0140	0,0004
RHD*10.00.01	DAU0.01	0,0119	0,0000	0,0000
RHD*03.01	DIIIa	0,0104	0,0190	0,0008
RHD*10.05	DAU5	0,0092	0,0050	0,0000
RHD*03.01.02	DIII.01.02	0,0040	0,0000	0,0000
RHD*09.01.02	DAR1.02	0,0039	0,0000	0,0000
RHD*10.04	DAU4	0,0034	0,0010	0,0000
RHD*09.01.00	DAR1.00 (WD 4.2)	0,0029	0,0810	0,0415
RHD*12.01	DOL	0,0023	0,0050	0,0000
RHD*49	DWN	0,0010	0,0020	0,0000
RHD*01.01	D48C	0,0010	0,0000	0,0000
RHD*01N.05	RHD*D-CE(3-7)-D	0,0009	0,0000	0,0019
RHD*01W.33	WD type 33	0,0009	0,0000	0,0000
RHD*01W.1	WD type 1	0,0008	0,1700	0,4200
RHD*01W.66	WD type 66	0,0006	0,0000	0,0000
RHD*01W.40	WD type 40	0,0005	0,0000	0,0000
RHD*37	DUC2	0,0003	0,0010	0,0000
RHD*05.01-10	DV.1	0,0003	0,0000	0,0000

RHCE*01.04.01 + RHCE*01.20.01/

OR

RHCE01.04.04 + RHCE*01.20.02

RHCE alleles		SIKKELCEL CHANG *et al.* 2020 n= 884	SIKKELCEL CHOU *et al.* 2018 n= 587	SIKKELCEL Van Sandt et al. 2023 n = 43	Present as ambiguity
RHCE*01	ce	0,2010	0,2730	0,1944	Yes
RHCE*01.20.01	ceVS.01	0,1390	0,1430	0,1667	Yes
RHCE*02	Ce	0,1170	0,1360	0,1389	Yes
RHCE*01.01	ce.01	0,2270	0,1930	0,1111	Yes
RHCE*03	cE	0,1020	0,1000	0,0694	Yes
RHCE*01.20.03	ceS	0,0450	0,0330	0,0694	Yes
RHCE*01.06.01	ceAG	0,4100	0,0430	0,0417	Yes
RHCE*01.20.09	ceVS.09	0,2700	0,0000	0,0417	
RHCE*01.04.01	ceAR	0,0006	0,0030	0,0417	
RHCE*01.02.01	ce.01.02	0,0300	0,0180	0,0278	
RHCE*04	CE	0,0011	0,0000	0,0278	Yes
RHCE*02:10	CeRN	0,0000	0,0017	0,0278	
RHCE*03.04	cEIV	0,0017	0,0000	0,0139	
RHCE*03.18	cE.18	0,0000	0,0000	0,0139	Yes
RHCE*02:09	CeCX	0,0000	0,0000	0,0139	
RHCE*01.20.02	ceVS.02	0,0310	0,0270	0,0000	Yes
RHCE*01.07.01	ceMO	0,0110	0,0140	0,0000	
RHCE*01.08	ceBI	0,0020	0,0030	0,0000	
RHCE*01.20.06	ceCF	0,0020	0,0009	0,0000	
RHCE*01.05.01	ceEK	0,0017	0,0003	0,0000	
RHCE*01.20.05	ceVS.05	0,0017	0,0000	0,0000	Yes
RHCE*01.03		0,0006	0,0000	0,0000	
RHCE*01.20.04.02	ceTI type 2	0,0006	0,0009	0,0000	Yes
RHCE*01.20.07	ceJAL	0,0006	0,0018	0,0000	
RHCE*02.08.01	CeCW	0,0006	0,0000	0,0000	
RHCE*02.22	Ce.22	0,0006	0,0000	0,0000	

Prediction of the most likely result based on expected haplotype

RHD* 01(/01W.150/01W.41) +09.02.01 OR RHD*05.04 + 09.01.02

RHCE*01.04.01 + RHCE*01.20.01 OR RHCE01.04.04 + RHCE*01.20.02

Predict the most likely haplotype

RHCE allele ISBT	RHCE allele common	HFA	LFA	ce	Often linked to RHD allele ISBT	Often linked to RHD allele	Population frequency†	Populatie	Anbibody	REF
RHCE*01.04.01	RHCE*ceAR	Hr–, hrS–	V+w, VS–	ew, c	**RHD*01**	RHD*POS		SCD		CHOU et al 2020
RHCE*01.04.01	RHCE*ceAR	Hr–, hrS–	V+w, VS–	ew, c	**RHD*09.01**	RHD*DAR		SCD	anti-e	CHOU et al 2020
RHCE*01.04.01	RHCE*ceAR	Hr–, hrS–	V+w, VS–	ew, c	**RHD*09.01**	RHD*DAR		US DONORS		Keller et al 2022
RHCE*01.04.01	RHCE*ceAR	Hr–, hrS–	V+w, VS–	ew, c	**RHD*09.01**	RHD*DAR	NR	TOTAL literatuurstudie		Sippert et al 2023
RHCE*01.04.01	RHCE*ceAR	Hr–, hrS–	V+w, VS–	ew, c	RHD*09.01.02	DAR1.2 (weak D 4.2.2)				
RHCE*01.20.01	RHCE*ce733G	hrB+, very weak to neg	V+, VS+	ep, cp	**RHD*01**	RHD*POS		US DONORS		Keller et al 2022
RHCE*01.20.01	RHCE*ce733G	hrB+, very weak to neg	V+, VS+	ep, cp	**RHD*01**	RHD*POS		BLACK		Zang et al. 2022
RHCE*01.20.01	RHCE*ce733G RHCE*ceVS.01	hrB+, very weak to neg	V+, VS+	ep, cp	**RHD*01/ RHD*09:03**	RHD POS / RHD*WD 4.0	Africa: 15.28%, America: 2.31%, East Asia: 0%, Europe: 0.30%	SCD	Anti-e	CHOU et al 2018
RHCE*01.20.01	RHCE*ce733G RHCE*ceVS.01	hrB+, very weak to neg	V+, VS+	ep, cp	**RHD*01/ RHD*09:03**	RHD*WD 4.0		SCD		Gaspardi, 2016
RHCE*01.20.02	RHCE*ce48C, 733G	hrB–	V+, VS+	ep, cp	**RHD*03.01**	DIIIa		SCD	Anti-D, anti-e	CHOU et al 2019
RHCE*01.20.02	RHCE*ce48C, 733G	hrB–	V+, VS+	ep, cp	**RHD*03.01**	DIIIa		SCD	Anti-D, anti-e	Gaspardi et al., 2016
RHCE*01.20.02	RHCE*ce48C, 733G	hrB–	V+, VS+	ep, cp	**RHD*09:01**	DAR		SCD		Gaspardi et al., 2016
RHCE*01.20.02	RHCE*ce48C, 733G	hrB–	V+, VS+	ep, cp	**RHD*09:03**	RHD*WD 4.0, DAR3		US DONORS		Keller et al 2022
RHCE*01.20.02	RHCE*ce48C, 733G	hrB–	V+, VS+	ep, cp	**RHD*09:03**	RHD*WD 4.0, DAR3		US DONORS		Gaspardi et al., 2016

Short read NGS
lacks,
as SSP,
locus and allele phasing information

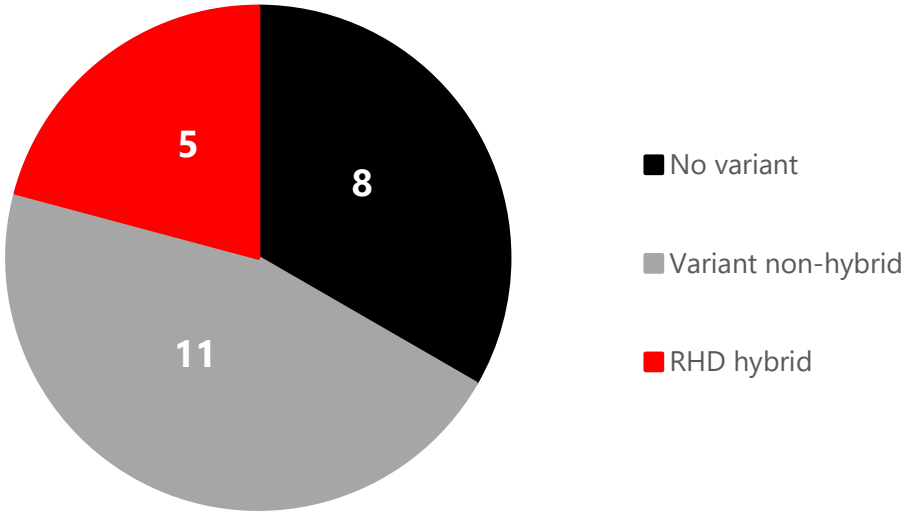
Different allele combinations remain possible

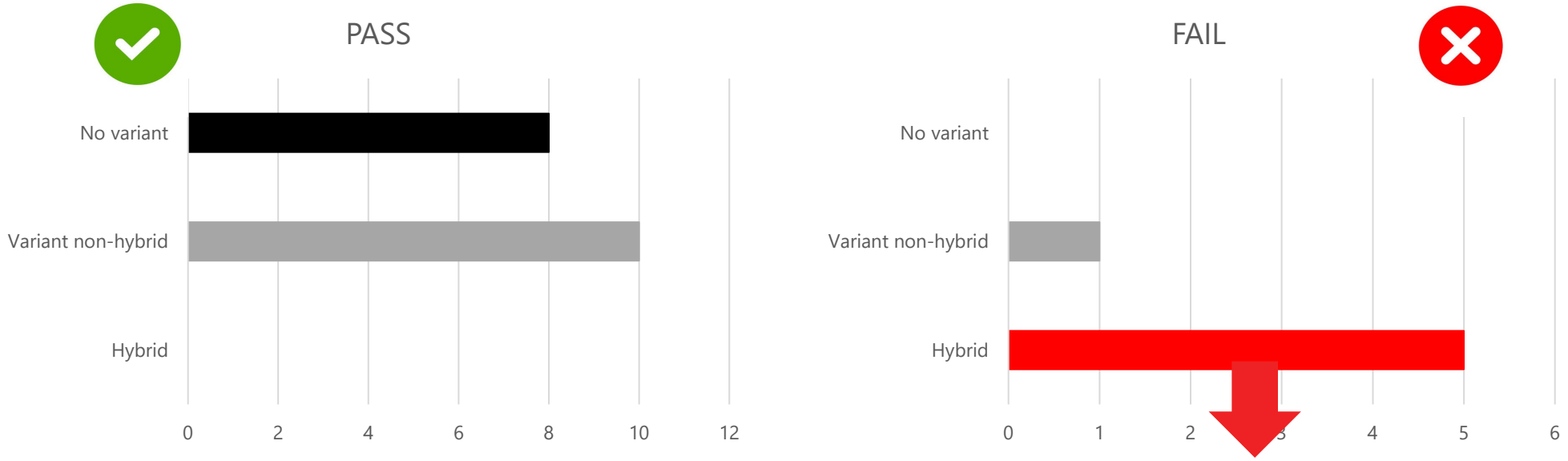
Prediction of the most likely result

Registration of RHD-RHCE variant haplotypes are very usefull.

Summary RHD

‘Selected’ cohort n= 24



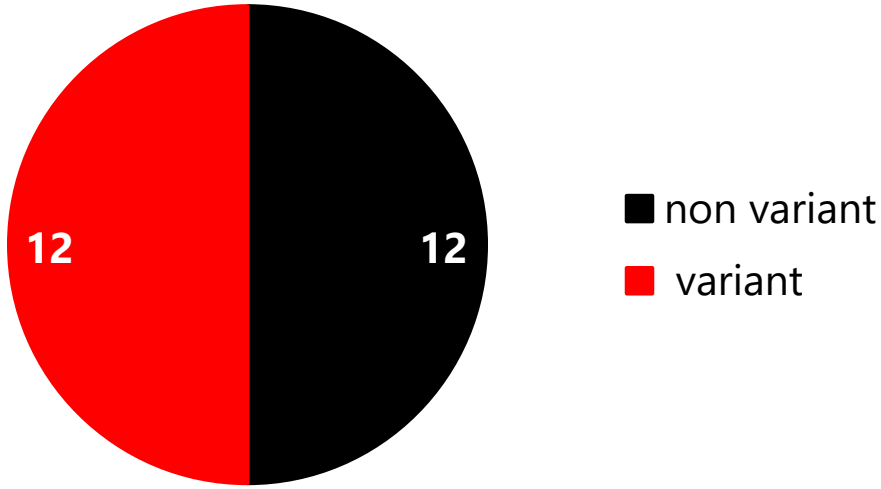


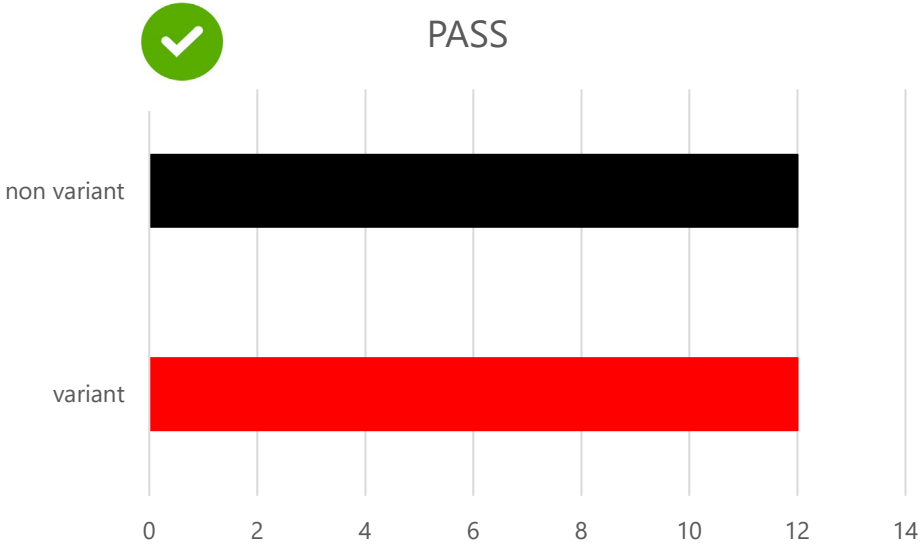
Exon – allel-loci balancing

Origin – expected haplo

Summary RHCE

‘Selected’ cohort n= 24





No failures

RHCE variants found:

RHCE*01.01, RHCE*01.04.01, RHCE*01.20.01, RHCE*01.20.02, RHCE*01.20.03, RHCE*01.20.04, RHCE*01.20.05, RHCE*01.20.06, RHCE*02.37, RHCE*03.18

Other bloodgroupsystems

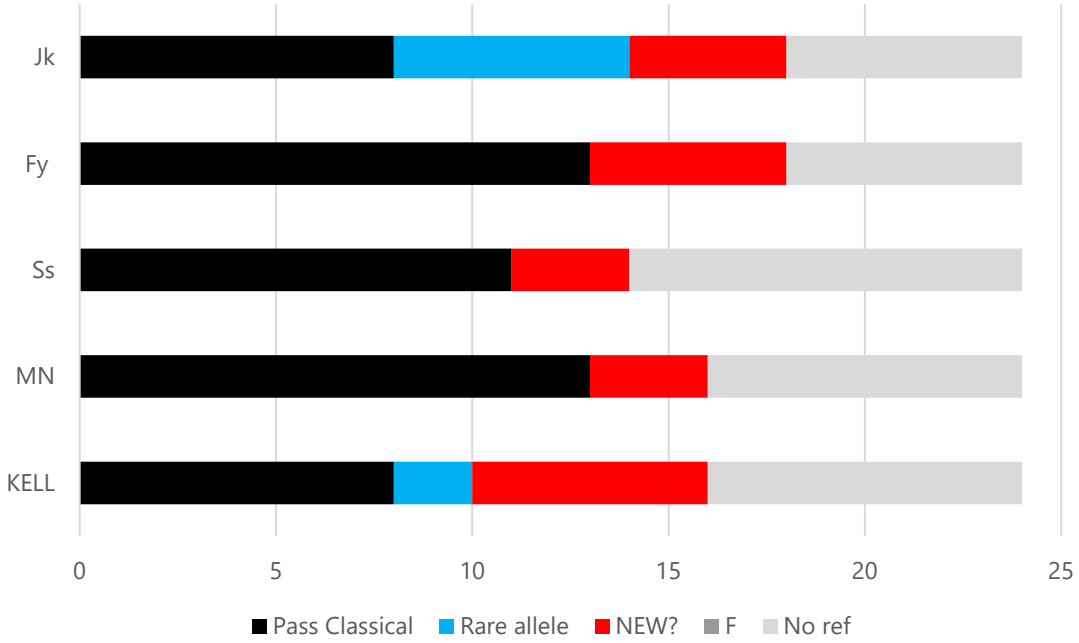
‘Selected’ cohort n= 24
Largely Sub-Sahara Africa origin

Not only RH has more variant alleles !

Kell, Kidd, Duffy, MNS

<u>Conclusion:</u>

- Classical alleles and variants such as FYGATA are identified correctly
- Rare alleles are identified
- Possible new alleles are identified: need some further investigation.



Conclusion

1) RBC-NGStype CORE

- Not Plug and Play: still needs (a lot of) manual editing, experience in interpretation, mainly due to incomplete or incorrect reference sequences.
- Struggels with HYBRID alleles
  - Shared exons between RHD and RHCE: crosstalk
  - Absence of locus and allele phasing makes it prone to error and leaves the result often ambiguous.

- Gives good results @ classical alleles and SNP variant alleles.
- Allows detection of rare and new alleles
- High throughput genotyping

→ 1st screening of patient samples / donor typing

More complex samples will probably benefit from LR NGS,
Not separating exons.
Making it easier to phase alleles and loci

THE COHORT IS WAITING

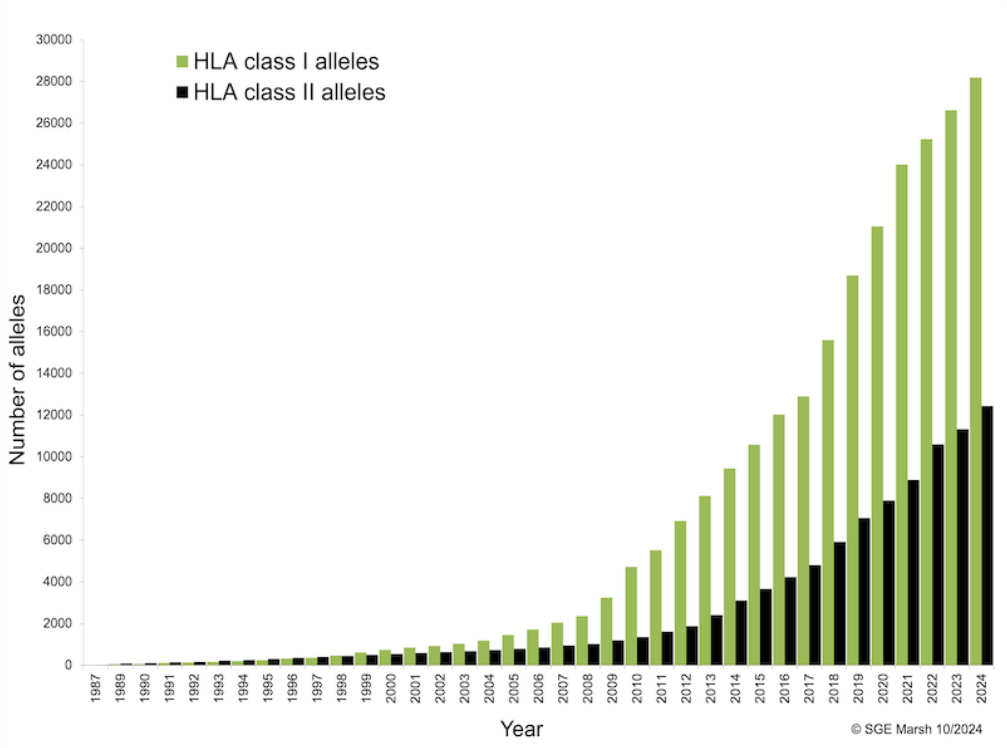
Conclusion

RBC antigen genotyping needs

a userfriendly RBC antigen database with compiled information // HLA antigen database, allele frequency.com

- cDNA
- gDNA
- Frequency ~origin
- Cathegorisation of frequnecy: H, L, U
- Associated haplotype RHD-RHCE
- Serology
- Statistics
- Allo immunisation risk / matching tool
- Info regarding regulation of gene expression.

- Align
- BLAST



Do not discriminate:
Adjust serology and genotyping to allow detection of variant alleles and adjust (when possible) transfusion policy.

Taking home message

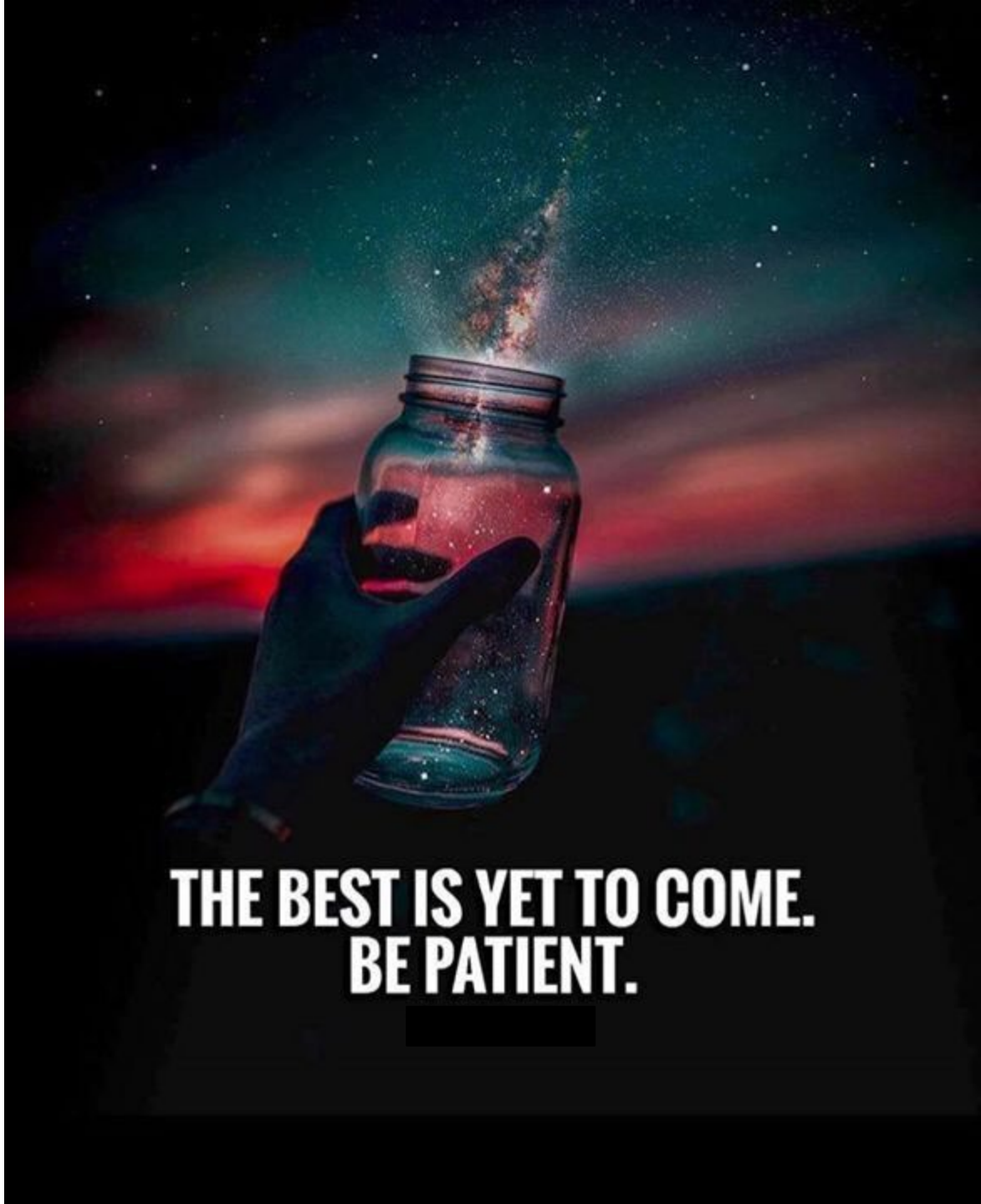
RBC Genotyping SSP – SSO- NGS

Do not just accept any result the interpretation table gives you, any result the software shows you.

Always be very critical

Does it match the serology?
Does it match the haplotype/ origin?
What are the limitations of the test?
Is there crosstalk / artefacts?

A lot is yet to be explored !
The best is yet to come !



Rode Kruis Vlaanderen

Dr. Stijn Van Landeghem
Dr. Linda Joosten

BL team

Dr. Ina Benoy
Dr. Johan Beert
Lien Veys
Steffi De Pelsmaeker
Johan Kerhofs

HILA team