



TEC533_TRUSIGHT HLA ASSIGN INSERTION ALIGNMENT AMBIGUITIES

Due to a limitation with the TruSight HLA Assign software, alleles that differ within an insertion cannot be resolved. This limitation is likely to become increasingly problematic in parallel with the ongoing rapid accumulation of HLA NGS data.

Impact and Presentation

Alleles that differ within an insertion will present as an ambiguity. An example of this is C*17:01:01/C*17:43, where TruSight HLA Assign is unable to resolve the insertion in exon 5.

5'UTR Exo Intron Exon 2 Intron 2 Exon 3 Intron 3	3	Exo	n 4 Intro	Exon	Intron 5			Intro E In
IMGT/C 3.37.0.1 2019-07-10	23612	sample C						
Base 2335 TTKCTGGCCYRGCTGTCCTGGYTGTCCTAGCTGTCCTWGCAGCTRTGRTSRC	CYGYTRTKATRYRT.	Start: 1, 5'UTR	1					
CDNA 297.3, Exon C A 2 1 R		Stop: 3356, 3'	JTR 170					
A	.CAGTG.	Allele l	Allele 2	CORE	EXONS	PHASE1	PHASE2	Differences
B	.TGACA.	C*07:02:01	C*17:01:01	0	0	0	0	
sample* C TIKCTGGCCYRGCTGTCCTGGYTGTCCT#GCTGTCCTWGGAGCTGTGGTCRC	CYGCTRTGATRYRT	C*07:02:01	C*17:43	0	0	0	0	
DPA1		C* <mark>07:02:55</mark>	C*17:01:11	0	0	1	1	Exon 3
DPB1 G TG T T A	C A GTG	C*07:27:01	C*17:48	0	0	1	1	Intron 7, Exon 3
DQA1 T CA C A G	T G ACA	C*07:51	C*17:11	0	0	1	1	Intron 3, Exon 2
DQB1		C* <mark>07:314:01</mark>	C*17:20	0	0	1	1	Exon 2
DRB1G03		C*07:736:01	C*17:38	0	0	1	1	Intron 7, Exon 7
DRB3		C*07:736:01	C*17:38	0	0	1	1	Intron 3, Intror
		C*07:15	C*17:23	0	0	2	2	Intron 3, Exon 3
108 (100)		C*07:76:01	C*17:22	0	0	2	2	Exon 2
	· · · · · · · · · · · · · · · · · · ·	C*07:744	C*17:16:01	0	0	2	2	Exon 3
		C*07:02:01	C*17:44	0	1	0	0	Exon 5
sample		C*07:02:01	C*17:47	0	1	0	0	Intron 6, Exon (
	99 Go	C*07:02:01	C*17:44	0	1	0	0	Intron 2, Intror
		C*07:02:01	C*17:47	0	1	0	0	Intron 2, Intror
A C G T A V 🖶 18 GGCTGT		C*07:02:01	C*17:44	0	1	0	0	Intron 3, Exon 5
Full Gene V Master V		C*07:02:01	C*17:47	0	1	0	0	Intron 3, Intror
		C*07:02:01	C*17:44	0	1	0	0	Intron 1, Intror

Fig 1. Presentation of C*17:01:01/C*17:43 in TruSight HLA Assign

As C*17:43 differs from C*17:01:01 at position 958, it should be possible to resolve this ambiguity.

cDNA	900	910	920	930	940		950	960	970	980
C*01:02:01:01	AGCCG	TCTTCCCAGC	CCACCATCCC	CATCGTGGGC	ATCGTTGCTG	GCCTGGCTG		CCTGGCTGTC	CTAGCTGTCC	TAGGAGCTGT
C*17:01:01:02				A-T	T	CATCCTGGC	TGT CCTGGCTG-	·		
C*17:01:01:03				A-T	T	CATCCTGGC	TGT CCTGGCTG-	·		
C*17:01:01:04				A-T	T	CATCCTGGC	TGT CCTGGCTG-	·		
C*17:01:01:05				A-T	T	CATCCTGGC	TGT CCTGGCTG-			
C*17:01:01:06				A-T	T	CATCCTGGC	TGT CCTGGCTG-	·		
C*17:01:01:07				A-T	T	CATCCTGGC	TGT CCTGGCTG-	·		
C*17:01:01:08				A-T	T	CATCCTGGC	TGT CCTGGCTG-	·		
C*17:01:01:09				A-T	T	CATCCTGGC	TGT CCTGGCTG-	·		
C*17:43				A-T	T	CATCCTGGC	TGT CCTGGCTG-	A		

Fig 2. Alignment of C*17:01:01 and C*17:43 with IMGT v3.38.0.

However, due to differences in the alignment algorithms between TruSight HLA Assign and IMGT, TruSight HLA Assign puts this polymorphism in the insertion. Because of this, this ambiguity cannot be resolved in TruSight HLA Assign.

The alignments differ as TruSight HLA Assign's alignment algorithm sees the sequence GGCTGTCCT(indicated below in pink) as the sequence replicated twice in the C*17:01:01 alleles and inserts the repeats(yellow) 3' of this at position 963, compared to IMGT's replication of TCCTGGCTG(blue) and insertion(yellow) at position 950.

 $S: \label{eq:sigma} S: \label{eq:sigma} S: \label{eq:sigma} Occas \label{eq:sigma} Occas \label{eq:sigma} Occas \label{eq:sigma} S: \label{eq:sigma} Occas \label{eq:sigma} S: \label{eq:sigma} Occas \label{eq:sigma} Occas \label{eq:sigma} S: \label{eq:sigma} Occas \label{eq:sigma} Occas \label{eq:sigma} Occas \label{eq:sigma} Occas \label{eq:sigma} Occas \label{eq:sigma} S: \label{eq:sigma} Occas \label{eq$



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TruSight HLA Assign:

IMGT:

C*17:01:01 AGCCGTCTTCCCAGCCCACCATCCCCAACTTGGGCCATCGTTTCTGGCCCAGCTG<mark>TCCTGGCTGTCCTGGCTG</mark>TCCTAGCTGTCCTAGGAGCTGTGGTCGCTGCTGTGA

C*17:43 AGCCGTCTTCCCAGCCACCATCCCCAACTTGGGCATCGTTCTGGCCCAGCTGTCCTGGCTGTCCTGGCAGTCCTAGCAGCTGTCCTAGGAGCTGTGGTCGCTGCTGTGA

Fig 3. Shaded alignment of C*17:01:01 and C*17:43 indicating the location of the sequences replicated and inserted.

				Replicated sequence Assign	Inserted sequence Assig	n
CDNA C*01:02:01:01 A C*17:01:01:02 - C*17:01:01:03 - C*17:01:01:04 - C*17:01:01:05 - C*17:01:01:06 - C*17:01:01:07 - C*17:01:01:08 - C*17:01:01:09 - C*17:01:01:09 -	900 910 AGCCG TCTTCCCAGC	920 930 CCACCATCCC CATCGTGGGGC 	940 ATCGTTGCTG GCCT TC, TC, TC, TC, TC, TC, TC,	GGCTG ATCCTGGCTGT CCTG ATCCTGGCTGT CCTG ATCCTGGCTGT CCTG ATCCTGGCTGT CCTG ATCCTGGCTGT CCTG ATCCTGGCTGT CCTG ATCCTGGCTGT CCTG	950 960 T CCTGGCTGTC CTA GCTG	970 980 SCTGTCC TAGGAGCTGT
C~1/:43 -		A-1	i	Inserted sequer	nce Replicated sequence IMGT	

Fig 4. Alignment in IMGT indicating the location of the sequences replicated and inserted.



Work around

If an ambiguity due to a difference in the insertion is suspected, check the alignment of the ambiguous alleles. Check the location of the insertion in TruSight HLA Assign by viewing the alignment of the reference sequences. To do this select References in the Views section of the Home Ribbon, then enter the names of the alleles to align in the box in the navigator and click filter. Any heterozygous positions and mismatches will be highlighted yellow.

							Ī				
5'UTR Exo Int	tron Ex	on 2 Intron 2 1	Exon 3 Intre	on 3	Exon 4	Intro	Exon	Intron 5		Intro	E Int
IMGT/C 3.37.0.1 2019-07	-10	<mark>229123012311</mark>	23212331	2341 23	5123612	3712381	sampl	le C			
Base 2335		CATCCCCAWCDTGGGCATCGTTKCTGGC	CYRGCTGTCCTGGYTGTCCT	AGCTGTCCTWG <mark>G</mark> AGCTRT)	GRTSRCYGYTRTKATRYRT.	AGGAGGAAGAGCTCA	Start	:: 1, 5'UTR 1			
cDNA 297.3, Exon C	A 2 1 R						Stop:	3356, 3'UT	R 170		
A		CATCCCCAWCWTGGGCATCGTTKCTGGC	CYRGCTGTCCTGGYTGTCCT	+GCTGTCCTWGGAGCTGT	GGTCRCYGCTRTGATRYRT.	AGGAGGAAGAGCTCA	Allel	le			
В		<mark>A</mark> . <mark>T</mark> <mark>T</mark>	. <mark>CA</mark>	<mark>.</mark> <mark>A</mark>	<mark>g</mark> . <mark>t</mark> <mark>g</mark> <mark>aca</mark> .		C*17:	01:01			
sample* C		<mark>A</mark> . <mark>T</mark> <mark>T</mark>	. <mark>CA</mark>	<mark>.</mark> <mark>A</mark>	<mark>g</mark> . <mark>t</mark> <mark>g</mark> <mark>aca</mark> .		C*17:	01:01			
DPA1		<mark>A</mark> . <mark>T</mark> <mark>T</mark>	. <mark>CA</mark>	<mark>.</mark> <mark>A</mark>	<mark>g</mark> . <mark>t</mark> <mark>g</mark> <mark>aca</mark> .		C*17:	01:01			
DPB1		<mark>A</mark> . <mark>T</mark> <mark>T</mark>	. <mark>CA</mark>	<mark>.</mark> <mark>A</mark>	<mark>g</mark> . <mark>t</mark> <mark>g</mark> <mark>aca</mark> .		C*17:	:01:01			
DQA1		<mark>A</mark> . <mark>T</mark> <mark>T</mark>	. <mark>CA</mark>	<mark>.</mark> <mark>A</mark>	<mark>g</mark> . <mark>t</mark> <mark>g</mark> <mark>aca</mark> .		C*17:	:01:01			
DQB1		<mark>A</mark> . <mark>T</mark> <mark>T</mark>	. <mark>CA</mark>	<mark>.</mark> <mark>A</mark>	<mark>g.t</mark> <mark>gaca</mark> .		C*17:	:43			
DRB1G03											
DRB3											
			sample								
			^	2335 ~	99 Go						
			ACCEPT A C G	ТА 🗸 🕂	18 TCCTGGCTGTCCT						
			Full Gene	✓ Master ✓							
			17:01:01,17:43		Filter						
						-					

Fig 5. By aligning the reference sequences for C*17:01:01 and C*17:43 we can see where TruSight HLA Assign has positioned the inserted sequence.

As Trusight HLA Assign cannot differentiate the sequences inserted, the insertion at position 2335 is not indicated as a mismatch in this alignment. TruSight HLA Assign does not show the inserted sequence for each reference allele. The sequence of the insertion for the sample will be displayed in the navigator.



Fig 6. Inserted sequence displayed in the navigator

Align the ambiguous alleles in IMGT and compare with the sequence observed.

cDNA	900	910	920	930	940		950	960	970	980
C*01:02:01:01	AGCCG	TCTTCCCAGC	CCACCATCCC	CATCGTGGGC	ATCGTTGCTG	GCCTGGCTG	T	CCTGGCTGTC	CTAGCTGTCC	TAGGAGCTGT
C*17:01:01:02				A-T	T	CATCCTGGCTGT	CCTGGCTG-			
C*17:01:01:03				A-T	T	CATCCTGGCTGT	CCTGGCTG-			
C*17:01:01:04				A-T	T	CATCCTGGCTGT	CCTGGCTG-			
C*17:01:01:05				A-T	T	CATCCTGGCTGT	CCTGGCTG-			
C*17:01:01:06				A-T	T	CATCCTGGCTGT	CCTGGCTG-			
C*17:01:01:07				A-T	T	CATCCTGGCTGT	CCTGGCTG-			
C*17:01:01:08				A-T	T	CATCCTGGCTGT	CCTGGCTG-			
C*17:01:01:09				A-T	T	CATCCTGGCTGT	CCTGGCTG-			
C*17:43				A-T	T	CATCCTGGCTGT	CCTGGCTG-	A		

Fig 7. Alignment of C*17:01:01 and C*17:43 with IMGT v3.38.0.

For further assistance contact CareDx tech support email: techsupport-global@caredx.com

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