Novel Allele or Incomplete Sequence? Classification of Mismatches in Extrapolated Regions by High Resolution Typing

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Introduction

While validating high resolution typing by nanopore sequencing for on-call deceased donor purposes, the discovery of novel alleles in a STAT scenario must be considered. Here, we present a sample that was typed by long read sequencing. The typing resulted in DQB1*03:03 + DQB1*05:02 with a mismatch in Exon 3, codon 117c for DQB1*03:03. A codon change from TGC→TGA with an amino acid change from cysteine→STOP was observed, predicting a novel null allele. This sample was reflexed to short read sequencing for confirmation and the typing resulted in DQB1*03:375N + DQB1*05:02. Differing results of DQB1 assignments prompted further investigation into the sequence analysis programs. In the long read software algorithm, extrapolated data is used to fill in the unknown portion of sequences. Whereas, in the short read software, the sequence is compared only against defined regions in the IMGT/HLA library. Genotype ranking in the long read analysis software places DQB1*03:375N as the 39th option for allele 1 due to mismatches in the extrapolated exon+ and intron regions. However, they are not considered true mismatches. For the short read analysis software, the sequence was a complete match for the known regions, exons 2 and 3, and the analysis software indicated the DQB1*03:375N. In this case, a novel null allele was obtained by long read nanopore sequencing. In our institution, novel alleles are confirmed through additional testing, however in a STAT scenario, confirmation would not be possible. For deceased donor typing entry into UNet, we would need to consider the clinical impact of this assignment. In order to eliminate the risk of donor-specific antibodies directed against this donor, our laboratory would list this donor as a DQ9 rather than a null allele.

Materials and Methods

DNA was isolated using the EZ1& 2 DNA Tissue and Blood kits from Qiagen. HLA high resolution typing was performed using the CareDx AlloSeqTx17 next generation sequencing platform and GenDx NGS-Turbo third generation sequencing platform.

Objectives

To highlight the importance of understanding the logic of the analysis software assignment, especially considering that numerous alleles in the IMGT database have incomplete sequences.

Results

NGSengine®-Turbo Analysis (long read sequencing)

DQB1	03:03[1]	05:02:01	DQ9	DQ5

Figure 1 — DQB1 typing results in NGSengine®-Turbo
A single mismatch in the exon+ region is indicated

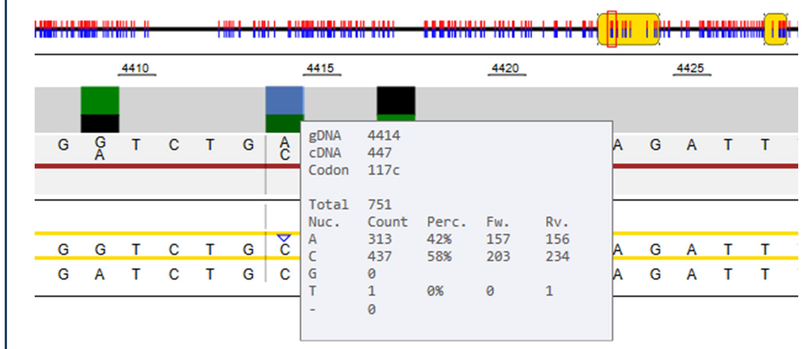

Figure 2 — Examination of exon+ mismatch
A nucleotide change from TGC→TGA and an amino acid change from cysteine→STOP described in Exon 3, codon 117c

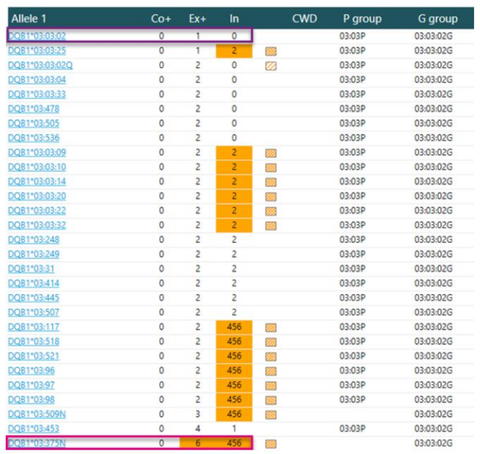

Figure 3 — Genotype ranking

AlloSeq™ Assign® Analysis (short read sequencing)

DQB1*03:375N (DQ"Blank")	DQB1*05:02:01 (DQ5)

Figure 4 — DQB1 typing results in AlloSeq™ Assign®

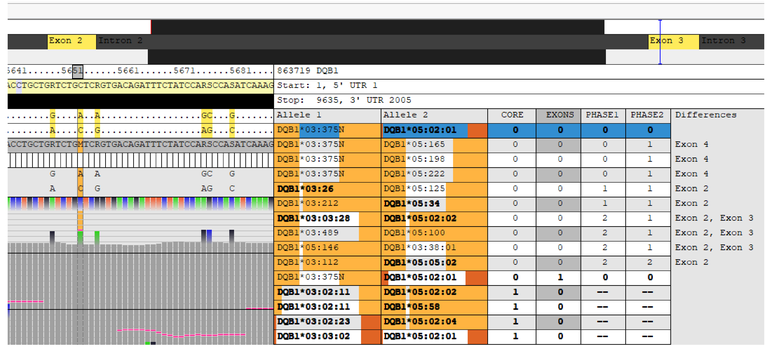

Figure 5 — Examination of results
Exons 2 and 3 are fully aligned with DQB1*03:375N reference sequence, the combination DQB1*03:375N + DQB1*05:02 was resulted with zero mismatches

Submission: HWS10100644 Full sequence data including introns obtained by long read sequencing was submitted to GenBank/IMGT HLA

The WHO Nomenclature Committee for Factors of the HLA System has officially named your sequence:

DQB1*03:375N

JH0163
GenBank: PV282397
Submission: HWS10100644

Conclusion

This case highlights the importance of understanding the logic of the analysis software assignment. Novel alleles of deceased donor typing results by nanopore sequencing challenges how we enter HLA typings into UNet. Additionally, completing undefined sequences through whole gene sequencing is integral in updating the IMGT/HLA libraries to fully define alleles, making HLA typing analysis more robust.