IDENTIFICATION OF DRB5*01:02 DROPOUT IN A LONG-RANGE PCR-BASED NEXT-GENERATION SEQUENCING HLA TYPING

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BACKGROUND:

HLA matching at allelic resolution is one of the most important factors affecting the outcome of allogeneic hematopoietic stem cell transplantation (HSCT) and reducing the risk of graft-versus-host disease and mortality. Therefore, an error-free HLA typing is critical for accurate donor-recipient compatibility to maximize HSCT success. HLA typing using next-generation sequencing (NGS) offers high-throughput and high-resolution capabilities, facilitating donor selection. However, long-range PCR NGS (LR-NGS) could yield allelic dropout, which is a well-known problem in PCR-based HLA typing methods, including Sanger sequencing-based high resolution HLA typing. Hybridization-capture NGS (HC-NGS), in comparison, can reduce the risk of allelic dropout and minimize erroneous typing although some challenges remain including phasing of polymorphisms.

In our lab, we perform HLA typing using both NGS techniques and examine their performance to determine an effective workflow incorporating both to address limitations of each assay.

CASES:

Here we describe DRB5*01:02 allele dropout tested by LR-NGS (MiaFora MFlex, Werfen), discovered in two unrelated HSCT donors.

Briefly, total genomic DNA was extracted from the two donor's peripheral blood, which were recruited by National Bone Marrow Donor registry (NMDP), using EZ1 advanced XL (Qiagen). HLA typing was performed on the two donors using the LR-NGS and analyzed by MiaFora v5.2, IMGT db v3.50. Results showed homozygous DRB5*01:01 for both samples (**Figure 1**). However, this call was discrepant with initial National Marrow Donor Program (NMDP) typing showing heterozygosity of DRB5*01:01 and DRB5*01:02.

To investigate this discrepancy, we tested the two donor samples by our 2nd method HC-NGS (AlloSeq Tx, CareDx) and analyzed by Assign v1.0.3, IMGT db v3.49. DRB5*01:01 and DRB5*01:02 were detected on both donor samples (**Figure 2**), which is consistent with initial NMDP typing. Meanwhile the two samples were sent to a reference lab and DRB5*01:02 was confirmed by AllType One Lambda along with TypeStream vTSV 3.0, IMGT db 3.53.0, a LR-NGS based method. The allele-specific DRB5*01:02 dropout by MiaFora MFlex is not due to poor DNA quality (**Figure 3**), but possibly due to either PCR thermal cycling not optimal for all primer sets used in a given PCR reaction or the specific allele fails to amplify because the gene-specific primer used in the long-range PCR amplification reaction is not annealing with the targeted allele due to uncovered mutations in the primer binding site, or preferential allelic-amplification causing the allelic imbalance, which can be inappropriately analyzed by the MiaFora software as background sequencing "noise" leading to the assignment of homozygosity with the preferentially amplified allele. Therefore, alternative methods can be used to confirm homozygous assignments in order to overcome such limitations (such as DRB5*01:2).

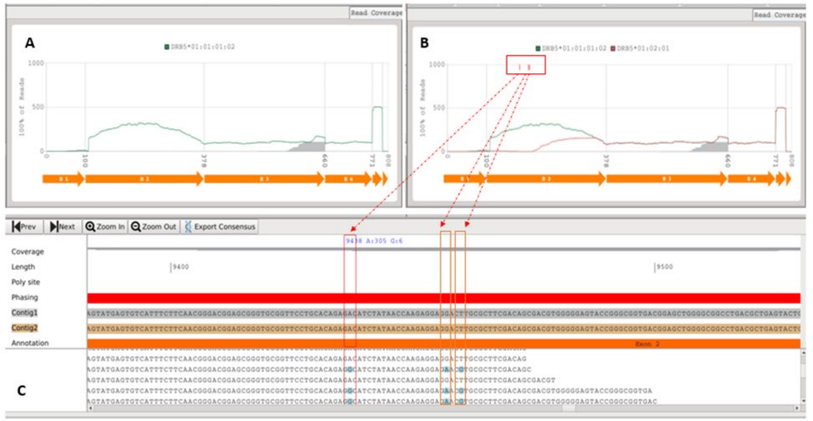

Figure 1. Representative DRB5 results on one of two HSCT donors tested by MiaFora MFlex. **A**. homozygous DRB5*01:01 was automatically called. **B**. DRB5*01:02 allele with extreme low coverage at the beginning of exon 2 failed to be identified. **C**. The three SNPs at the beginning of exon 2 showed very low reads on the DRB5*01:02 allele.

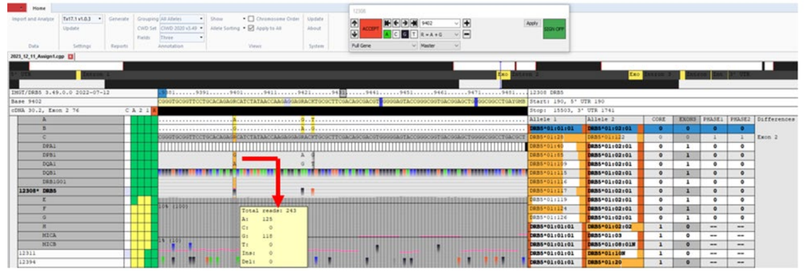

Figure 2. Representative DRB5 results on one of two HSCT donors tested by AlloSeq Tx. Both DRB5*01:01 and DRB5*01:02 alleles were detected.

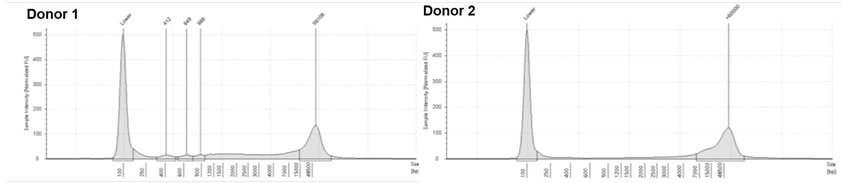

Figure 3. DNA quality evaluation by Fragment Analyzer indicating good DNA quality on the two HSCT donor samples.

CONCLUSIONS:

The allelic dropouts observed here uncover the limitation of using LR-NGS as a standalone method for clinical HLA testing. Our findings suggest that the homozygosity of certain HLA loci (such as DRB5*01:02) need to be confirmed by alternate methods (such as HC-NGS) to prevent allelic dropouts.

REFERENCES:

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