NGS SUPERPOWERS II: NEW CAPABILITIES FOR CHALLENGING SAMPLES

Ellia Gomes¹, Efi Melista1, Krisztina Rígó2, Libor Kolesa2, Milena Vrana3, Peter Meintjes1

¹ Omixon Inc., USA, ² Omixon Biocomputing Kft, Hungary, ³ Institute for Hematology and Blood Transfusion, Czech Republic.

Introduction

Human leukocyte antigen (HLA) genes are the most complex and polymorphic system in the human genome, making their genotyping very challenging in the clinical setting. In recent years, the continuous and partial characterization of new alleles has significantly increased the level of ambiguities using traditional genotyping techniques (SSO, SBT), as a result requiring reflexive testing, sometimes extensive. Next Generation Sequencing (NGS) is an advanced method able to provide unambiguous results and eliminate the need for reflexive testing in the clinical laboratories.

We aim to use the Holotyper HLA kit and the unique consensus generation algorithm of HLA Twin to resolve previously ambiguous or problematic samples to show how easy it is to detect and interpret novel alleles and unambiguously confirm null alleles.

Methods

All the samples presented here were typed using Holotyper HLA Kit and sequenced on the Illumina MiSeq. The data was analyzed with Omixon’s HLA Twin software (v2) and IMGT/HLA database v3.28 on customers’ sides and then independently reanalyzed and confirmed by Omixon’s R&D experts with the latest software version and IMGT/HLA database v3.28 for consistency.

Novel Allele Detection – Sample 1

Sample 1 is an example of a novel allele. During the routine typing of a bone marrow donor, using allele technology (SBT), the result C*02:02:02:01+06:19 was obtained. However, the other result C*02:02:02:01+06:02:01:01 was more probable but it contained one mismatch (position 570 in Exon 2). It has shown up that it might be a new mutation (Fig. 1). The sample was re-tested by NGS and Holotyper HLA and confirmed the formerly observed mutation in exon 2, while another novel SNP was detected in exon 1 (Fig. 2). This is not unusual because legacy technologies, such as SBT, pose certain limitations such as a more limited gene coverage, resulting in missed information.

In HLA Twin the naming convention of novel allele candidates contains the C nucleotide. With the Amino Acid Track function ON, you easily inspect that the new mutation results in change in the amino acid sequence while the bottom track displays the re-alignment of the reference closest matching allele in the IMGT database. The differences of the novel sequence and the reference sequence as well as how many reads support them at the position in question are clearly visible for each sample (Fig. 3 and Fig. 4).

Discussion

These examples of novel and null alleles are only a small sample of the powerful tool that is NGS and how clinical labs use Holotyper HLA and HLA Twin to tackle some of their most challenging samples in a way that was not previously possible.

As shown in sample 1, SBT technology is limited to certain exons only. In this case routine SBT typing has revealed only one new polymorphism while NGS detected two different novelities in exons changing the amino acid sequence of the HLA molecules. These new SNPs will have an impact on the protein structure of the final molecule and on the specificity of its binding grooves. Additionally, sample 2 demonstrates the power of HLA Twin’s consensus generating algorithm in being able to determine the entire genomic sequence of not only new alleles, but also partially characterized ones. This will be an important hold that the community will move towards as intrinsic information may reveal alternative splice proteins or regions that affect regulatory and expression levels of the resulting HLA proteins. Similarly, accurate and correct resolution of null alleles is a critical aspect in the HLA community as well as detecting long insertions/deletions in exons shorter than the HLA sequence. By changing the visualization mode into a short read mode with collapsed reads it is possible to investigate the sequence of each read and determine whether the insertion is sufficiently covered while excluding the common allele as a possible result.

Conclusion

Due to the benefits of NGS, HLA typing laboratories worldwide are switching their laboratory workflows to adopt the NGS technology. Both registries and clinical research laboratories can benefit from the superpowers of this new technology. Holotyper HLA that includes the HLA Twin software provides the right solution for high resolution HLA typing of organ transplant patients and hematopoietic stem cell donor registries.