COMPARISON OF TWO NGS-BASED METHODS FOR HIGH-RESOLUTION HLA TYPING

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Abstract. Objectives. Our aim was to implement and compare two NGS-based methods for high-resolution HLA typing. **Methods.** 20 DNA samples from external quality assessment (Instand e.V., Germany) were genotyped using both (1) Omixon Holotype HLA 24/7 Kit for library preparation, sequencing on Illumina MiSeq instrument and analyzed with Omixon HLA Twin 4.8.1 software and (2) Thermo Fisher One Lambda AllType™ FASTplex™ NGS 11 Loci Flex Kit for library preparation, sequencing on Illumina MiSeq instrument and analyzed with Thermo Fisher One Lambda TypeStream Visual™ 2.0 software. **Results.** Omixon Holotype HLA 24/7 Kit library preparation takes more than 12 hours (at least two working days) with approximately 3 hours hands-on time and approximately 35% of the protocol is single-tube. One Lambda AllType™ FASTplex™ library preparation takes less than 7 hours (one working day) with 1.5 hours hands-on time and approximately 70% of the protocol is single tube. Both kits required 17 hours for sequencing with Illumina MiSeq Nano v2 kit. One Lambda AllType™ FASTplex™ library preparation protocol is much faster (can be completed in single working day) and typing results can be obtained in approximately 25 hours compared and much more convenient with 1.5 hours hands-on time and 70% of protocol performed in single tube, where Omixon Holotype HLA 24/7 protocol can be practically completed in 48 hours, and is much more laborious with double hands-on time and 35% of protocol being single tube. For Omixon qPCR library quantitation is critical as improper quantitation due to inefficient adapter ligation results in severe underclustering and analysis failure. **Conclusion.** One Lambda AllType™ FASTplex™ protocol is much faster and much more convenient to use compared to Omixon Holotype HLA 24/7 protocol. With all improvements, however, the sample-to-results time with 2nd generation of NGS is unlikely to become less than 24 hours, which limits its application only to live donors and we expect that 3rd generation sequencing will be able to bring high resolution HLA typing to cadaveric donors in emergency situations to take advantage of MatchMaker and PIRCHE II algorithms matching in solid organ transplantation.