AUTOMATING HOLOTYPE HLA ON TBG’S DX-A™ ROBOT

Elia Melista1, Tina Yu2, Tunde Vago1, Willy Hsu3, Peter Meintjes3, Tim Hague1

1 Omixon Biocomputing Kft
2 TBG Biotechnology Corp.
3 Omixon Inc

Introduction

HLA typing by Next Generation Sequencing (NGS) is increasingly being adopted for high-resolution HLA genotyping. Holotype HLA is a commercially available NGS-based HLA genotyping product for up to 11 HLA loci that has been implemented in more than a dozen clinical labs worldwide. To ensure reproducibility and repeatability across technical staff and to reduce the possibility of human error, pre-PCR and post-PCR steps of the protocol can be automated on liquid handling robots due to the flexibility of Holotype HLA.

We present the results of Holotype HLA 24/7 and 24/11 as automated on the DX-A liquid handling system by Texas BioGene (TBG). The DX-A is a cost-effective system with a small physical footprint that only needs a laptop to operate (Picture 1), and can provide accurate, consistent results eliminating the risk of human error.

Methods

We performed a Holotype HLA 24/7 run using the automated protocol designed specifically for the TBG DX-A pipetting system on 24 DNA samples that were previously typed manually with the Holotype HLA kit. Each sample was amplified at 7 loci (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DPB1, HLA-DQB1). The samples were sequenced on an Illumina Miseq using the 2x250 bp chemistry and the results files were analyzed using Omixon’s HLA Twin 1.1.4.2 software.

The genotyping results generated from the automated protocol on TBG DX-A were compared to previously determined genotypes from a manually performed Holotype run.

Results & Discussion

The Holotype HLA protocol in an HLA clinical laboratory to be as efficient as possible - to reduce technician time and opportunities of human error. Additionally, the flexibility of the assay makes it possible to perform the whole protocol manually within about 5 hours of hands-on time (HoT). When using the scripts provided for the TBG DX-A the total HoT is reduced from 5 hours to 1.45 hours. With the pre-PCR and post-PCR automation this new protocol can save 3 hours of valuable time and reduce cost when processing Holotype HLA either for 7 or 11 loci. The detailed steps of the protocols, manual and robot hands-on times for the 24/7 kit can be found in Graph 1.

The total time required (including LR-PCR and other incubation times) is about 14.5 hours. During this pilot project, the scripts for the Holotype HLA 24/11 workflow we developed, but the validation is yet to be completed. The hands-on times for the 11 loci kit measured during initial testing are displayed in Graph 2.

The steps that have been automated include: input DNA dilution, LR-PCR setup (Picture 2), amplicon quantitation and normalization, per-sample pooling of HLA loci, library preparation and final pooling. We used both single channel and multichannel (8 channel) pipetting options in the protocol. To leverage the power and accuracy of TBG DX-A automated liquid handling system, we optimized the robot with the least possible number of pipetting steps.

The robot run times of both HLA Holotype automated protocols can be seen in Table 1.

Conclusion

Automation of Holotype HLA 24/7 and 24/11 kits on the TBG DX-A was successfully implemented on the TBG DX-A. The protocols that were developed reduced the hands-on time significantly, while the total time of the workflow remains the same as for manual processing.

Most importantly, the TBG DX-A eliminates variation and errors among technicians reducing inter-run variability to offer consistency and accuracy in genotyping results.

The TBG DX-A uses a simplified programming language that makes it easy to implement, and easy to modify for different lab throughputs, while being among the most cost effective liquid handling solutions on the market today. All of the above make the TBG DX-A a suitable liquid handling system for use with the easy and streamlined Holotype HLA for any HLA lab.