AUTOMATING HOLOTYPE HLA™ ON THE AGILENT BRAVO NGS WORKSTATION
LIQUID HANDLER & VALIDATION FOR ROUTINE HLA GENOTYPING

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Introduction

HLA typing by Next Generation Sequencing (NGS) is increasingly being adopted for high-resolution HLA genotyping due to the technological advancements that allow complete characterization of the HLA genes. For almost all combinations of alleles, the genotyping results present with no ambiguity, eliminating reflexive testing. Holotype HLA is a NGS-based method for HLA typing being adopted by HLA laboratories over legacy technologies such as SSO and Sanger SBT for its increased accuracy, resolution, repeatability and high-throughput nature. The workflow is easy-to-follow with minimal hands-on time on manual implementation (Figure 1).

However, 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 to provide the most reliable results within a 3-day turnaround time from sample to answer.

Here we present the development of a protocol that streamlines multiple modules of the NGS process on the Agilent BRAVO Option B for pre-PCR steps and Option A for the post-PCR steps. The difference between the two robot configurations is that Option B includes a plate/tip box stacker, while Option A does not.

Results

Here we present results of the development of methods for both pre- and post-PCR steps of the Holotype HLA workflow on the Agilent Bravo liquid handler that was performed at our HLA laboratory. Without automation, the total hands-on time required for completing the entire protocol is more than 4 hours for experienced users, and is reduced by more than an hour with the current automated methods for both pre- and post-PCR steps. It’s worth noting that three steps in the workflow (gDNA preparation, HLA-specific master mix preparation and amplicon normalization) are still under development in our laboratory and once they are finalized the hands-on time is expected to be less than 2 hours for the entire protocol. Table 1 shows the hands-on time (HoT) and total time (TAT) when comparing manual vs automated processing of a Holotype HLA 24/11 kit as they currently stand.

Since one of the aims of the development was to be able to process different numbers of samples on the liquid handler depending on the lab’s varying workload, methods were also created for processing 8 and 16 samples, all at 11 loci. Interestingly, we found that the times change only minimally, allowing us to scale up and down freely without affecting our turnaround time and without increasing the user’s hands-on time (Table 2). Specifically, some steps, such as the Class I and Class II amplification plate setup and the amplicon quantitation preparation remain unaffected by the number of samples being processed (Figure 3).

We also consider that if we were to process as many as 96 samples at a time, the 96-tip pipetting head of the BRAVO would be able to achieve the process with great time efficiency.

Discussion

HLA typing by NGS is increasingly being implemented by laboratories worldwide due to the highly accurate, unambiguous results it produces, eliminating the need for reflexive testing. At the same time, optimizing the workflow and throughput of the technology to accommodate an HLA laboratory’s work load to achieve accuracy and reproducibility while minimizing the risk of user error is equally important.

In our laboratory, several modular methods on the Agilent BRAVO were developed to automate the Holotype HLA kit by Omixon. Currently, the automated methods have decreased the user’s hands-on time as much as 1 hour and 40 minutes. Ongoing current development of additional steps (gDNA preparation and amplicon normalization) will further decrease the hands-on time. Further bringing it to less than 3 hours from beginning to end while the validation of the automated methods for our clinical routine is ongoing. It’s noteworthy that scaling up or down to the adjusted lab workload at any time is effectively feasible with an insignificant increase to the user’s hands-on time when using the BRAVO (Figure 4) due to its high capacity 96-tip pipetting head allowing us to process any number between 8 to 96 samples within a similar timeframe.

Additionally, several QC steps that were used during different steps of the workflow confirmed that the samples processed on the robot produce the expected results and that the expected data are of high quality, making the Agilent Bravo NGS Workstation a very suitable partner in efficient HLA typing by NGS using Holotype HLA in under 48 hours from sample to result.

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