MULTI-CENTER STUDY FOR AUTOMATING HOLOTYPE HLA™ ON A BIOMEK 4000

Efi Melista[1*], Kim McGowan[2**], Doreen Sese[2], Sharon Adams[3], Deborah Chen[3**], Tünde Vágó[4], Eric McCloskey[1], Zach Smith[5***], Shilpa Parakh[5], Peter Meintjes[1]

[1]Omixon Inc., Cambridge, MA, [2]Yale University, New Haven, CT, 3NIH Clinical Center, Bethesda, MD, [4]Omixon Biocomputing Kft, Hungary, [5]Beckman Coulter Life Sciences, Indianapolis Indiana
Primary author of the presented results, **Primary contributors of data for the results,*Primary author of the Biomek 4000 method*

Introduction

NGS-based (Next Generation Sequencing) HLA typing can offer accurate and unambiguous genotyping results with minimal user intervention eliminating the requirement for reflexive testing.

Holotype HLA is a powerful 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, the elimination of human and random error is a desirable attribute of any laboratory test and the implementation of a liquid handling system can significantly reduce both sources of error in an NGS workflow.

The development of a protocol that streamlines multiple modules of the NGS process was the goal of this multi-center study.

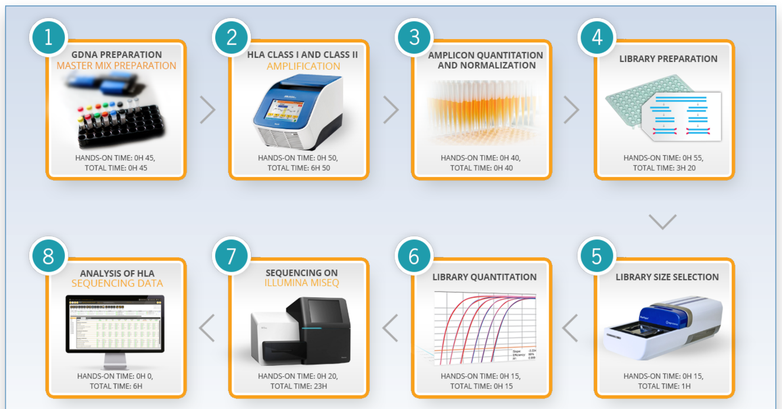

FIGURE 1 - Holotype HLA workflow

Methods

The Beckman Coulter Biomek 4000 automated liquid handler (Fig. 2) is a small footprint solution for pre- and post-PCR processes in NGS-based HLA typing. During development, the protocol was optimized through comparison of QC tests to determine the effectiveness and success of the reactions of each step (i.e. amplification, fragmentation, adaptor ligation, pre- and post-size selection). Twelve (12) samples were run in duplicate for eleven (11) loci; each set was processed twice (2 independent runs) with the Holotype HLA 24/11 kit by Omixon to assess the accuracy, repeatability and reproducibility of the automated Holotype HLA solution. Three centers participated in this study: Yale University, the NIH Clinical Center and Beckman Coulter Inc.

The runs that were processed by Beckman Coulter's facility were used solely to develop and fine tune the method to the reagents' properties as well as to achieve optimal pipetting capacity by the robot. The data produced by Yale University and the NIH Clinical Center were used to calculate the relevant metrics mentioned above.

All automated pre- and post-PCR steps of the Holotype HLA workflow were tested and the sequencing data was analyzed with HLA Twin (the software component of Holotype HLA).

FIGURE 2 - The Biomek 4000 Liquid Handler by Beckman Coulter

Display does not represent the actual Biomek 4000 configuration. Biomek Automated Workstations are not intended or validated for use in the diagnosis of disease or other conditions.

Results

All steps that can be automated in the workflow of Holotype HLA have been implemented on the Biomek 4000. The steps include the gDNA preparation, master mix preparation and amplification setup on pre-PCR, and on post-PCR, the amplicon quantitation preparation, amplicon normalization and the entire library preparation.
Table 1 shows all Holotype HLA steps with detailed hands-on (HoT) and total times (TAT) for both manual and automated processing while Figure 3 is a graphical representation of the hands-on time only that is required for manual vs Biomek 4000 processing. The hands-on time is noteworthy as it is reduced by more than two hours whereas there is an increase of about two hours in the total time. This is resulting from the use of slow pipetting speeds and pipette mixing to ensure optimal transfer of viscous reagents at lower volumes. Also in some cases the single vs 8-channel pipetting tool is used to reduce the reagent dead volume.

	HoT 24/11 Biomek 4000	TAT 24/11 Biomek 4000	HoT 24/11 Manual	TAT 24/11 Manual
Step 0 gDNA preparation	0:05	0:23	0:20	0:20
Step 1 HLA Amplification Master Mix preparation	0:10	0:50	1:00	1:00
Step 2 Class I & Class II plate setup + PCR	0:05	6:50	0:40	7:10
Step 3 Amplicon Quantitation and normalization	0:20	2:00	0:50	0:55
Step 4 Library Prep	0:50	3:48	0:55	2:50
Step 5* Pippin Prep Size Selection	0:10	0:55	0:10	0:55
Step 6* Library Quantitation - qPCR	0:10	1:05	0:10	1:05
Step 7* MiSeq preparation and load	0:20	23:20	0:20	23:20
Step 8* Data Analysis	0:00	6:00	0:00	6:00
Total (including sequencing and data analysis)	**2:10**	**45:11**	**4:25**	**43:35**

**Steps 5, 6, 7 and 8 are always manual so there's no difference in timing*

TABLE 1 - Detailed HoT and TAT time comparison for manual run vs Biomek 4000

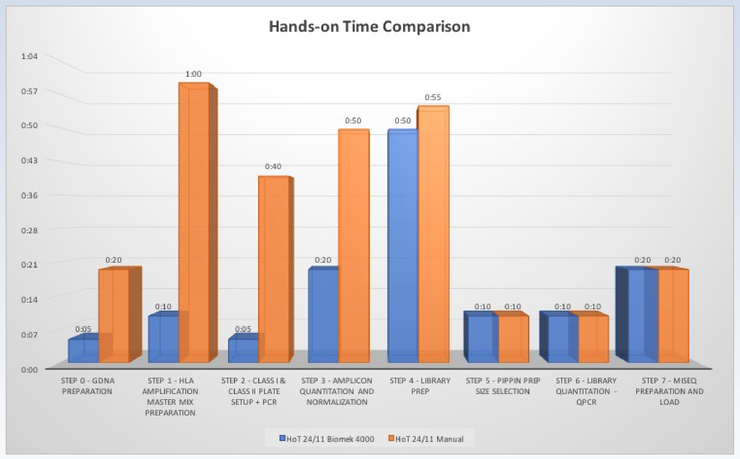

FIGURE 3 - Hands-On Time comparison between manual processing and on Biomek 4000

Results continued

Genotype assignments from all sites were assessed by allele-level matching to the 2nd field for intra-run variability, inter-run variability, and total accuracy (Figure 4). All non-concordant samples were interpreted for quality, and re-analyzed using the HLA Twin software; samples that remained inconsistent with their reference assignment were assigned as mismatches.
The metrics are presented cumulatively for both sites (Yale University and NIH Clinical Center). Intra-run variability was measured at 2.4% with 97.6% of the results matching perfectly. Similarly, 97.6% of the genotypes were concordant with the reference data provided by the laboratories. Finally, the inter-run run variability was a bit lower than the intra-run one at 1.7% with 98.3% of the duplicates within a run matching as expected.

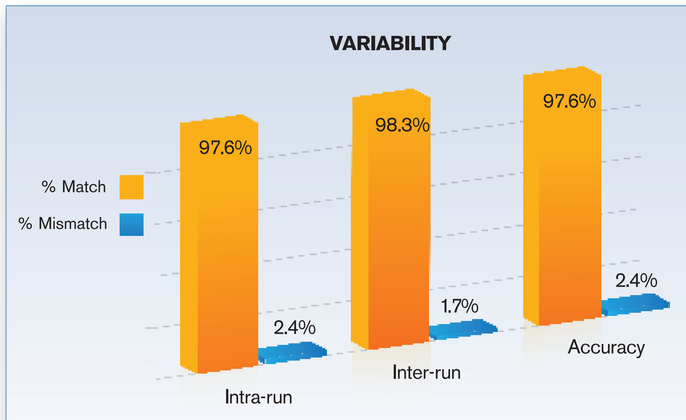

FIGURE 4 - Intra- run and inter-run variability as well as accuracy were measured at 2nd field concordance based on reference genotypes provided by each site.

Discussion

The primary goal of this study was to develop a method capable of providing highly accurate and reproducible HLA genotypes using Holotype HLA on the Biomek 4000 by Beckman Coulter. Additionally, an equally important goal was to develop a method that minimizes the user hands-on time and as a result minimizes the risk of errors during the NGS run. Collaboratively the scientists from Beckman Coulter and Omixon were able to produce a method that reduces the hands-on time by almost 3 hours in the 48-hour total time required, significantly reducing the risk of human error (4.5h vs 1.75h HoT).
Data was obtained from two HLA labs that have already validated Holotype HLA for manual implementation (Yale University and NIH Clinical Center). All data was analyzed using the same version of Omixon HLA Twin software (v2.1.4) and IMGT/HLA database v3.28, and assessed for reproducibility, repeatability and accuracy via comparison with reference allele assignments. The variability observed (2.6% for intra-run and 1.7% for inter-run) was caused by either a locus dropout, or by an insufficient amount of sequencing data produced for a certain locus leading to an incorrect result. In the case of insufficient data present, HLA Twin's Quality Control metrics flag the locus for investigation by the user.

Conclusions

Overall, the results obtained demonstrate that the method achieves highly reproducible results for all 11 loci across runs, with minimal intra-run variability, and high accuracy based on the reference data made available by each site for the samples processed.
We can conclude that high-throughput NGS-based HLA typing is achievable in under 48 hours from DNA to Genotype using the Beckman Coulter Biomek 4000 modular-based NGS protocol for Holotype HLA 24/11.

Acknowledgements

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