AUTOMATING HOLOTYPE HLA™ ON EPPENDORF'S EPMOTION LIQUID HANDLER AND VALIDATING IT FOR ROUTINE HLA GENOTYPING

Authors: Vera Siffnerova [1], Kirk Mantione [2], Efi Melista[3]

1. Omixon Biocomputing Ltd., Hungary 2. Eppendorf North America, USA 3. Omixon Inc., USA

Introduction

Due to its ability to fully characterize HLA genes despite their dense variability and sequence homology, Next Generation Sequencing (NGS) has become the most widely implemented method in HLA laboratories over the past years. NGS methodologies provide lower ambiguity rates, significantly reduce reflexive testing, and when used optimally, also improve cost efficiency.

Holotype HLA is a commercially available NGS-based HLA genotyping product for up to 11 HLA loci. Due to its workflow flexibility (Figure 1), labs of any throughput can easily implement NGS. To reduce the risk of human error and improve reproducibility and repeatability, many of the labs wish to implement it on automated liquid handlers. This is easily achievable for Holotype HLA due to the simplicity of the protocol, which was designed in a clinical lab with automation in mind.

Here we present the development of an automation method with multiple modules of the NGS process on the epMotion 5075t for all of the pre-PCR and post-PCR steps of Holotype HLA.

FIGURE 1: Holotype HLA workflow

Methods

The Eppendorf epMotion 5075t (Figure 2) is a multi-purpose liquid handling workstation, an ideal walk-away system for labs that demand high efficiency, accuracy and reproducibility. This makes it a great solution for the NGS workflow. For the implementation of Holotype HLA 24/11 (v3), the epMotion 5075t was equipped with a thermal module, gripper tool, single-channel and multichannel dispensing tools (50 uL and 300 uL).

The Holotype HLA automated workflow was split into four sub-methods, one pre-PCR (Long-Range PCR setup) and three post-PCR (amplicon quantitation, normalization, per-sample pooling of HLA loci and sample purification, library preparation and final pooling), table 1. Figure 3 shows the deck layouts for the corresponding sub-methods.

The run was performed in the Eppendorf North America facility to develop and finetune the methods to the reagents' properties as well as to achieve the optimal pipetting capacity of the robot.

All automated pre- and post-PCR steps of the Holotype HLA workflow were tested and the data were analyzed using HLA Twin v3.1.3, the software component of Holotype HLA.

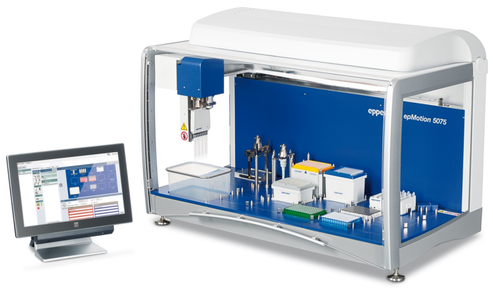

FIGURE 2: epMotion 5075t Liquid Handler by Eppendorf

Automated sub-methods	Protocol Steps *safe stopping points	On deck times (Does not include incubation times)	Tip type	Tip consumption
Method 1 (deck layout Fig. 3-A)	LR-PCR Setup*	56 min	50 uL	170
Method 2 (deck layout Fig. 3-B)	Amplicon Quantitation	65 min	50 uL 300 uL	241 38
Method 3 (deck layout Fig. 3-C)	Amplicon Normalization	60 min (depending on number of dilutions)	50 uL	up to 360 (depending on number of dilutions)
	Per-sample Pooling			
	Amplicon Cleanup*			
Method 4 (deck layout Fig. 3-D)	Fragmentation*	20 min	50 uL	129
	End Repair*			
	Adapter Ligation*			
	Library Pooling*			

TABLE 1: An overview of the automated sub-methods of the Holotype HLA 24/11 kit configuration on the epMotion 5075t

FIGURE 3: Deck layouts for each of the 4 sub-methods for the Holotype HLA 24/11 kit configuration. A. LR-PCR set-up, B. Amplicon quantitation, C. Amplicon normalization, per-sample pooling, and Amplicon cleanup, D. Library preparation

Results

Here we present the results from the development of the automation methods for the pre- and post-PCR steps of Holotype HLA 24/11 on the epMotion 5075t workstation.

Without automation, the total hands-on time required for completing the entire protocol is a little over three hours for experienced users, which is reduced by more than an hour when using the automated methods of the epMotion 5075t for both pre- and post-PCR steps. When comparing total times, they are slightly longer when using the epMotion platform as opposed to performing the assay manually, however, the difference is negligible in the context of the entire workflow. Table 2 shows the detailed hands-on (HoT) and total times (TAT) for both manual and automated processing of a Holotype HLA 24/11 kit configuration.

	HoT 24/11 epMotion 5075t	TAT 24/11 epMotion 5075t	HoT 24/11 Manual	TAT 24/11 Manual
Step 1 LR-PCR setup	0:02	6:56	0:15	6:15
Step 2 Amplicon Quantitation	0:05	1:05	0:20	0:20
Step 3 Amplicon Normalization	0:02	0:15 (dependent on the number of dilutions)	0:20	0:20
Step 4 Library Prep	0:02	1:40	0:20	1:40
Step 5* Library Size Selection	1:20	1:20	1:20	1:20
Step 6* Library Quantitation	0:15	0:15	0:15	0:15
Step 7* MiSeq Preparation and Load	0:20	19:20	0:20	19:20
Step 8* Data Analysis	0:00	3:00	0:00	3:00
Total (including sequencing and data analysis)	2:06	32:51	3:10	32:30

*steps 5, 6, 7 and 8 are always manual so there is no difference in timing

TABLE 2: Hands on Time (HoT) and Turnaround Time (TAT): Manual vs on epMotion 5075t Liquid Handler for a Holotype 24/11

For validation of the methods, 16 DNA samples were processed on the epMotion 5075t for all the protocol steps. All samples had been previously processed manually and their sequencing results were compared to the results obtained with the automated methods. Additionally, one of the samples was run in duplicate to test reproducibility.

The tested samples displayed a genotypic concordance rate of 99.62% in comparison to the expected typings. Essentially, all results matched with the exception of a single DQA1 locus that was reported as a novelty due to high background noise (unrelated to the automated methods). The sample that was run in duplicate was reproducible both in genotypes and data quality for all loci.

In addition to genotyping concordance, the data quality of all samples was reviewed using HLA Twin's quality control (QC) metrics. One of the more relevant criteria that are built in HLA Twin are QC metrics to detect sample contamination, whether that is from mis-pipetting (in manual processing) or from cross-over pipetting in automated methods.

These QC metrics along with 20 additional ones that reflect the overall data quality were within expected ranges.Figure 4 shows a high level overview of the sample quality as shown in HLA Twin.

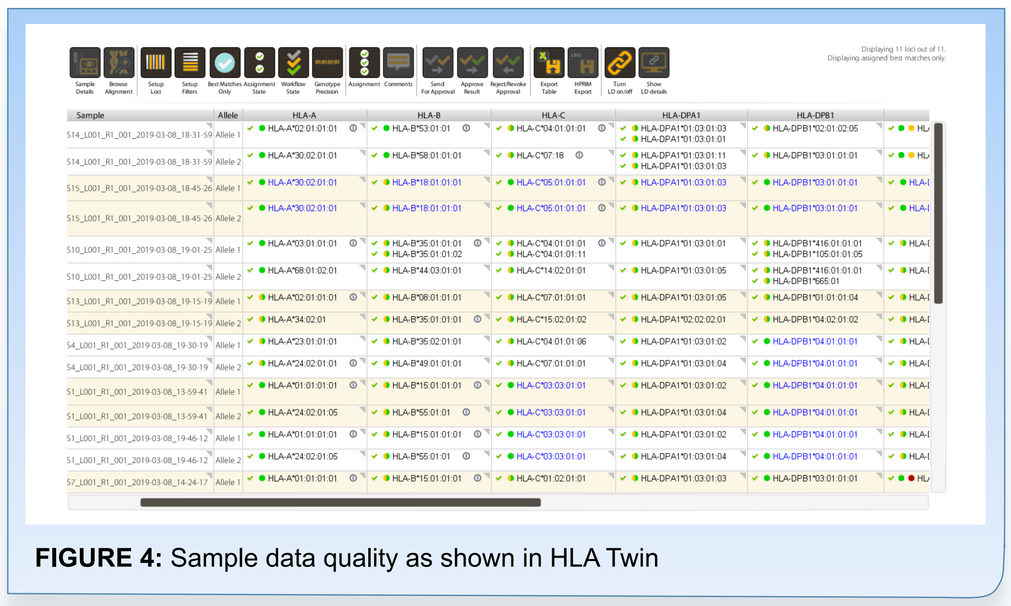

FIGURE 4: Sample data quality as shown in HLA Twin

Conclusion

Eppendorf's epMotion 5075t can be positioned as an ideal automation solution for small to medium sample throughput labs with maximum flexibility for varying sample numbers up to 24 and high adaptability for the Holotype HLA automated workflow for both pre- and post-PCR steps. Implementing automation with the epMotion 5075t liquid handler methods reduce the hands-on time by over one hour, down to 2 hours and 6 minutes in the Holotype HLA workflow, providing an end-to-end solution for any HLA lab. Most importantly, it eliminates the inter-technician and inter-run variability offering consistency, accuracy, and eliminates the risk of human pipetting error.

The hands-on time is significantly reduced, while the total processing time of the workflow is slightly increased in comparison to the manual processing, however the increase is insignificant (21 min) and does not affect a typical lab's workday scheduling or result reporting times.

Finally, the epMotion control software has a user-friendly interface to guide the operator through the workflow, including placement of the labware, tools, and reagents, making it an attractive solution to new automation users and beyond. The instrument is very easy to program and can provide accurate, consistent results eliminating the risk of human error in a wide range of laboratories.