APPLICATION NOTE 426

Automated library preparation for HLA testing using the Omixon Holotype HLA™ kit (24/11) on the Eppendorf ep*Motion*® 5075t

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Abstract

Producing robust DNA libraries from precious samples is a key requirement for many next generation sequencing (NGS) applications. Automated library preparation minimizes sample loss and reagent usage and can also help eliminate a source of variability. Eppendorf has collaborated with Omixon, to develop an automated workflow for the Omixon Holotype HLA kit (24 samples/11 loci) on the Eppendorf ep*Motion* 5075t automated liquid handling system. The data generated from the automated protocol show that this kit can be readily implemented on this automation platform to generate consistent, high quality libraries for sequencing up to 24 samples.

Introduction

For transplant medicine, HLA typing methods need to be continually improved. The use of next generation sequencing is becoming increasingly more accepted in lieu of traditional Sanger sequencing. In addition to allowing multiplexing of samples, NGS provides a greater level of information in this highly polymorphic portion of the genome. Automated liquid handling offers consistency and accuracy by eliminating the risk of human pipetting error including inter-technician and inter-run variability. The Omixon Holotype HLA kit workflow including the pre- and post-PCR steps was successfully automated using the ep*Motion* 5075t.

Holotype HLA is a commercially available NGS-based HLA genotyping product for up to 11 HLA loci. Due to its flexibility, most laboratories can easily implement NGS library preparation. This is achievable for Holotype HLA due to the simplicity of the protocol, which was designed with automation in mind.

The ep*Motion* software has a user-friendly interface to guide the operator through the workflow, including placement of the labware, tools, and reagents. Before the run starts, an optical sensor verifies that all labware is correctly placed. The ep*Motion* can be equipped with accessories for plate mixing, on-deck incubations, and magnetic bead separation. The automated platform allows for maximum walk away time.

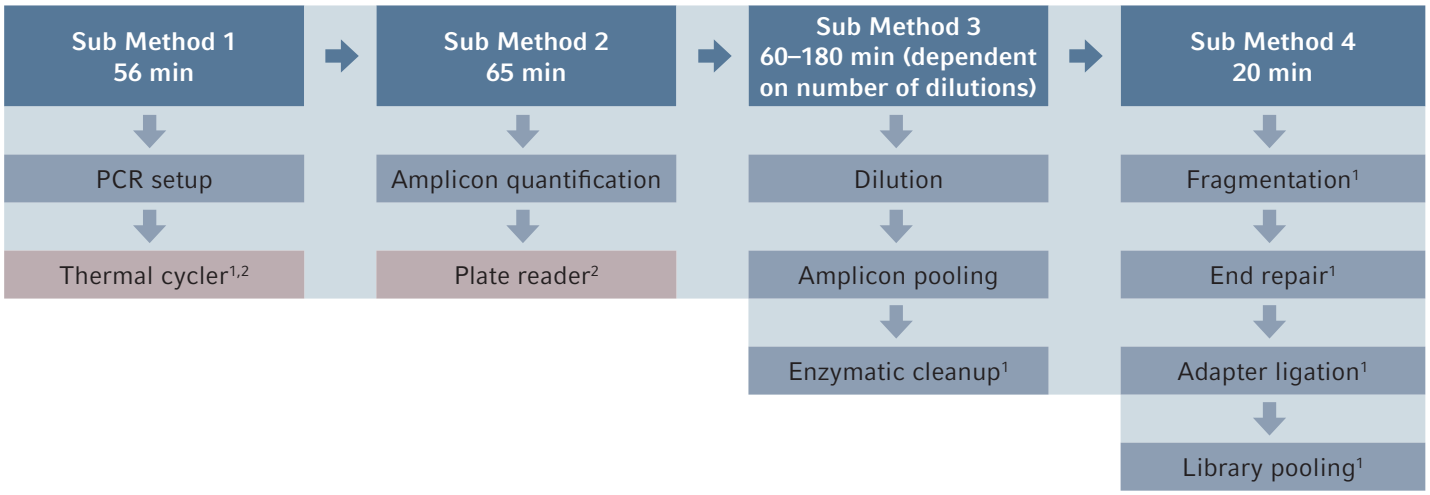
Figure 1: Eppendorf ep*Motion* 5075t automated liquid handling system

The ep*Motion* 5075t was designed for small to medium sample throughput with maximum flexibility for varying sample numbers (Figure 1). The ep*Motion* 5075t is capable of significantly reducing the hands-on time for technicians in the Holotype HLA workflow, providing an end-to-end solution for any HLA lab.

Materials and Methods

Automated liquid handling was performed using the ep*Motion* 5075t equipped with a thermal module, gripper tool, single-channel and multichannel dispensing tools (50 µL and 300 µL). The Omixon Holotype HLA kit for 24 samples and 11 loci (version 3.0) was employed to generate DNA libraries for sequencing. For testing the methods, samples were processed on the ep*Motion* 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. Samples were sequenced using a MiSeq® and the read length was between 140–147 bp after quality trimming and the sequencing depth averaged 150 bp. In addition to genotyping concordance, the data quality of all samples was reviewed using Omixon HLA Twin's quality control (QC) metrics.

The method starts with the long-range PCR set up. After PCR, the amplicons were quantified using the Promega QuantiFluor® kit. The quantified PCR products were then diluted appropriately, pooled, and enzymatically purified. Finally, the libraries were prepared and pooled before being subjected to an off-deck bead purification step. Altogether, 4 separate methods were performed on the 5075t. The long-range PCR is performed off deck and requires about 6.5 h to complete. The library preparation steps also have off-deck handling requirements for the fragmentation, end repair, and adapter ligation. These reactions are performed in a thermal cycler and consist of incubations lasting 25, 35, and 20 minutes respectively.



[1] Safe stopping points
[2] Off deck

Figure 2: Workflow for the Omixon Holotype HLA kit. The steps are grouped into 4 sub-methods on the ep*Motion*. Timing and tip consumption are based on 24 samples.

Results and Discussion

The total on-deck processing time for the methods was about 3 h (Figure 2). The same samples prepared manually would have a total hands-on time of about 4.5 h. In addition to the greater than 30 % time saving, by automating most of the liquid handling, the risk for human pipetting error was eliminated. The automated procedure yielded final library with a concentration of 6.7 nM. The post-size selection fragment size of the purified libraries averaged 492 bp (Figure 3). These results were in accordance with recommended amounts to successfully sequence.
One of the more relevant criteria for HLA typing is the quality of the downstream analysis. To further analyze the quality of the libraries a sequencing run was performed using a MiSeq with 2x150 bp with an analysis on HLA Twin. When compared with the known genotypes of the samples, the final libraries prepared on the ep*Motion* show excellent concordance (99.44 %). All results matched except for a single DQA1 locus that was reported as a novelty due to high background noise (unrelated to the automated methods). One sample that was run in duplicate was also reproducible both in genotypes and data quality for all loci. The results also show a consistent balance with the average allele ratio of 53.2 % allele 1 to 46.8 % allele 2. When compared to the same samples prepared by manual pipetting, the allele ratio of the loci sequenced indicated that there were no differences introduced by the procedure indicating successful automation. Taken together the yield, fragment lengths, and sequencing concordance thus all support the efficacy of the ep*Motion* platform for automating the Omixon Holotype HLA kit for this critical step of the HLA screening workflow.

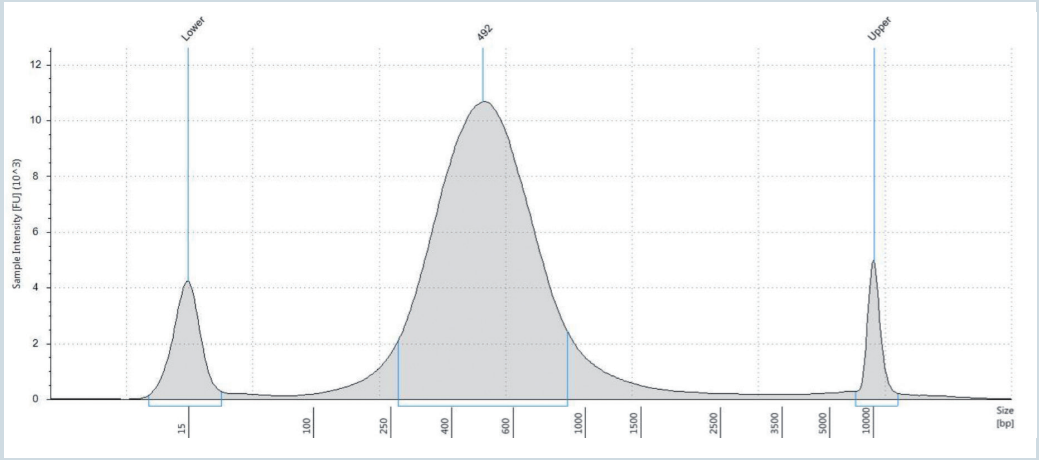


Figure 3. Tapestation® electropherogram of purified pooled samples prior to sequencing.

Conclusion

The increased demand for high-quality NGS libraries in high-throughput laboratories has necessitated the development of robust, automated methods for library preparation. Omixon has collaborated with Eppendorf to automate the Holotype HLA 24/11 Kit on the Eppendorf ep*Motion* 5075t. The experimental data shows that the automated method performs within the specifications at the input amount tested. The highly flexible and modular automated solution for NGS library preparation will allow laboratories to easily scale up processes without sacrificing quality in the process.

Ordering information*

Description	Catalog No.
ep*Motion*® 5075t	5075006022
Thermal module on position C2	960002181
TS 50 Dispensing Tool	960001010
TM 50-8 Dispensing Tool	960001044
TS 300 Dispensing Tool	960001028
TM 300-8 Dispensing Tool	960001052
Gripper	960002270
Thermoblock PCR 96 OC	5075751666

Ordering information

Description	Catalog No.
Thermoadapter PCR 96 (2)	960002199
Reservoir rack 3	5075754070
epT.I.P.S.® Motion, 50 µL, filtered	0030014413
epT.I.P.S.® Motion, 300 µL, filtered	0030014456
ep*Motion*® Reservoirs, 30 mL	960051009
Eppendorf twin.tec® PCR Plate 96, skirted	0030129512
Thermorack for 24 safe lock tubes 1.5/2.0 mL	960002075
Mastercycler® X50a	6313000018

* Please note that all products in this list are Research Use Only (RUO)

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