Automation of multiplexed 11-Loci HLA NGS Illumina library preparation on the *HLA PRO* Instrument

Matthew McFaul*, Loni Townsley*, Harry O. Lopez, Dennis McIlhany, Dan Ma, Peter Brescia, JJ Chen
Transplant diagnostics business of Thermo Fisher Scientific Inc., West Hills, CA, USA
**Authors contributed equally to this project*

Abstract

Purpose: The goal of this project is to combine the power of a highly multiplexed HLA-typing NGS assay with the precision and flexibility of the HLA PRO Instrument, a general-purpose liquid handling pipettor to automate Illumina library preparation for NGS HLA-typing assays.

Methods: We developed amplicon purification and library preparation scripts for the HLA PRO instrument and tested the performance of these scripts on multiple instruments, with various number of samples being processed, and investigated reagent stability (over time and upon freezing/defrosting). The performance of the HLA PRO instrument and script workflows were assessed at multiple stages – amplicons yield, NGS library quality, and finally HLA locus coverage and typing accuracy.

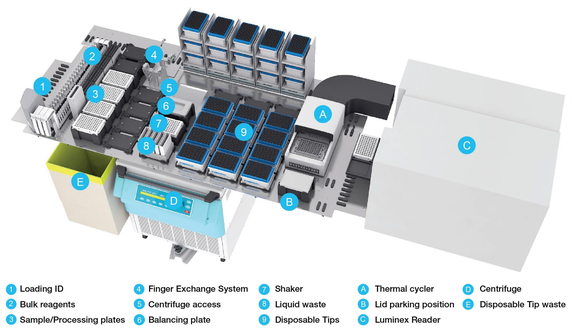
Results: The HLA PRO instrument demonstrated robust performance for NGS library preparation across many conditions – various throughputs, multiple kit uses, and different instruments.

Introduction

Next generation sequencing (NGS) based HLA-typing is a powerful tool that offers unparalleled high quality typing resolution. One of the main challenges with NGS assays are long and sensitive workflows, often spanning multiple days with many steps that require careful attention. To streamline this procedure and alleviate labor demands, we have automated the workflow for an 11-loci (HLA-A, -B, -C, -DRB1, -DRB3,4,5, -DPA1, -DPB1, -DQA1 and -DQB1) NGS library preparation assay on HLA PRO, built on the framework of the Tecan Fluent pipettor instrument.

The HLA PRO can fully automate library preparation for 8 to 96 samples with unique barcodes and common sequences for subsequent amplification at the ends of DNA fragments to be sequenced on the Illumina sequencing platforms.

Figure 1. *HLA PRO* deck layout for amplification and library preparation of NGS libraries and system overview.



System overview					
Robotic arms	Capable of using 2 robotic arms simultaneously				
Deck check	Utilize a step-by-step deck loading procedure to help customers load correct labware and reagents, comparing to the expected deck layout and highlight any discrepancies.				
Robotic gripper arm	The long Z axis on the gripper arm enable the access to below deck; automatic Finger Exchange System with gripper fingers – eccentric and long eccentric				
Door locks	Doors (hood and centrifuge) lock protect process from free access				
Liquid handling performance (volume range)					
Tip type	10µL	50µL	200µL	350µL	1000µL
Volume precision	0.5µL, 10µL	1.0µL 50µL	10.0µL 200µL	10µL 200µL	10µL 1000µL
CV	≤6.0% ≤1.0	≤4.0% ≤0.3%	≤2.0% ≤0.2%	≤2.0% ≤0.5%	≤2.0% ≤0.3%
Accuracy	±9.5% ±2.0%	±8.0% ±0.5%	±2.0% ±0.5%	±2.0% ±0.5%	±3.0% ±0.0%

Materials

Sample Preparation

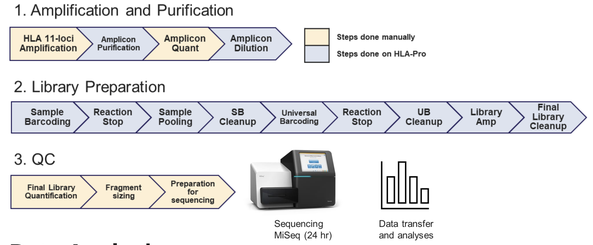
Reference DNA samples were selected from genetically diverse, well-characterized samples from the UCLA/Exchange program and the International Histocompatibility Working Group (IHWG). The extracted genomic DNA samples were characterized with Qubit and TapeStation.

Methods

Workflow

NGS libraries for 8, 24, and 96 samples were prepared using the 11-loci HLA NGS kit on the HLA PRO platform following the hybrid workflow in **Fig. 2**. Multiple runs of the same 24 samples were performed across 2 different HLA PROs on 5 non-consecutive days. The final sequencing libraries were analyzed using the Agilent TapeStation for fragment size and Qubit for concentration. The libraries were sequenced on Illumina sequencing platforms.

Figure 2. Workflow on *HLA PRO* platform using the 11-loci HLA NGS typing kit



Data Analysis

The barcode balance, locus coverage, and allele balance were analyzed for each sequencing run to ensure performance. HLA typing was generated with the TypeStream Visual (TSV) software, and sample concordance was determined with independently verified reference typing.

Results

The automated library preparation generated high quality libraries with concentrations greater than 5 ng/µL. Sequencing depth was greater than 200 for key exons, which along with exon 1 coverage to resolve additional ambiguities, resulted in 11 HLA loci typing that was >99% concordant to the third field across all samples tested.

Multiple freeze thaws for library preparation reagents had no impact on library quality or sequencing results, indicating that this process can be adapted for various use configurations.

Table 1. Results of 8, 24, and 96 sample library preparation on *HLA PRO*

Metrics/Sample Size	8	24	96
Amplicon Concentration (ng/µL)	> 5	> 5	> 5
Avg. Library Size (bp)	737	697	723
Avg. Library Concentration (ng/µL)	16.2	21.2	16.2
Barcode %CV	14%	19%	29%
Avg. Exon 2 Depth Uniformity	22%	24%	37%
Avg. Exon 2 Coverage per Locus	> 200	>200	>200
Avg. Exon 2 Allele Balance	>0.2	>0.2	>0.2
3rd Field Concordance	100%	100%	100%

Conclusions

The HLA PRO Instrument and workflow scripts automate multiplexed-amplicon purification and library preparation to deliver high quality libraries for 11 HLA loci and exon 1 in under 8 hours. This flexible platform allows for library preparation for as few as 8 or as many as 96 samples and creates libraries that are fully compatible with Illumina sequencers.

This workflow dramatically reduces hands on time for NGS library preparation, while consistently generating high-quality and high-resolution HLA typing data.

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