HLA PRO Automation of 11 Loci NGS Assay Library Preparation For Ion Torrent

Dan Ma, Loni Townsley, Matt McFaul, Harry O. Lopez, Dennis McIlhany, Peter Brescia, JJ Chen
Transplant diagnostics business of Thermo Fisher Scientific Inc., West Hills, CA, USA

Abstract

Purpose: The aim is to automate workflow for 11-loci HLA NGS Ion Torrent library preparation to reduce hands-on operations and labor demands using the HLA PRO system.

Methods: Two scripts were tested: one for the automated purification of amplicons containing the targeted HLA genes via PCR, and the other for fully automating the processes of amplicon dilution, sample barcoding, pooling, universal barcoding, amplification, and final library purification steps.

Results: The HLA PRO can generate a multiplexed HLA library comprised of sequencer-ready fragments derived from amplified genomic DNAs. The automated HLA PRO significantly decreases the hands-on time required to prepare the library for sequencing on the Ion Torrent system.

Introduction

Sequencing the human leukocyte antigen (HLA) region offers essential information for transplant diagnosis. HLA typing via next-generation sequencing (NGS) offers remarkable flexibility and achieves high resolution. Some laboratories may encounter challenges in adopting NGS because of its heightened complexity and demanding labor needs. 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 upon the framework of the Tecan Fluent pipettor instrument. The HLA PRO can fully automate library preparation for 8 to 48 samples with unique barcodes and common sequences for subsequent amplification at the ends of DNA fragments to match the Ion Torrent system.

Materials and methods

Sample Preparation

The 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 will be quantified using Qubit and quality will be assessed with TapeStation.

Test Methods

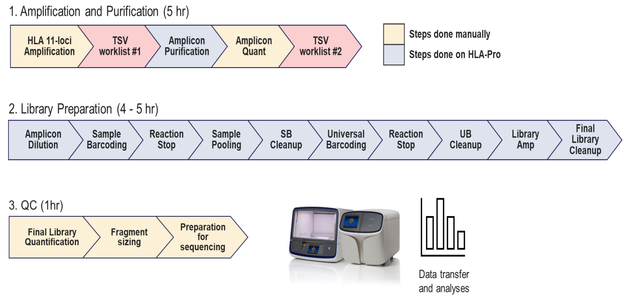
Libraries that are composed of various number of samples will be prepared using the 11-loci HLA NGS kit, followed by amplicon dilutions and automated library preparation runs on the HLA PRO, and sequenced on the Ion Torrent platform. The final sequencing libraries will be evaluated using the Agilent TapeStation for fragment size and Qubit for concentration. Each run will be assessed for barcode balance, locus coverage, and allele balance to ensure performance, and data from all runs will be combined to confirm HLA typing accuracy, compared to verified reference typing.

Data Analysis

FASTQ data files obtained from the Ion Torrent sequencer will be imported and analyzed using TypeStream Visual (TSV) NGS Analysis Software using the appropriate catalog and library file. The accuracy of the HLA typing results obtained will be compared with the known HLA reference typing to obtain concordance at Field-3 level using the LB Clopper-Pearson method.

Each HLA allele typing is treated as a separate data point, derived from locus-specific sequenced reads. TSV software filters these reads, aligns them to reference alleles from the IMGT HLA Sequence Database, and assigns an allele through independent alignment. All loci typing data are analyzed together for concordance, with target gene amplification, library preparation, and sequencing conducted across all loci. Concordance is defined by at least one matching allele between experimental and reference typing.

Workflow



Results

HLA PRO amplicon purification script effectively purified PCR amplicons, yielding high concentrations with minimal variability.

To evaluate the efficacy of the automated amplicon purification process performed by an eight-channel pipettor, identical amplicons were pooled and underwent purification using the HLA PRO system. Quantitative analysis has shown that the system effectively purified the amplicons, yielding high concentrations with minimal variability.

Figure 1. Workflow diagram illustrating the automated amplicon purification process using the HLA PRO system.

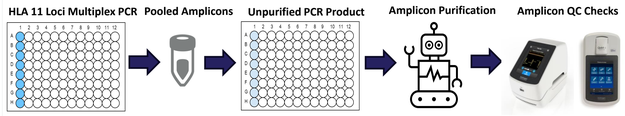
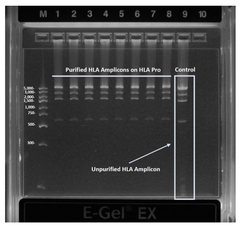
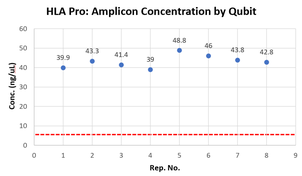


Figure 2. Image of a 2% E-gel showing purified HLA amplicons processed by HLA Pro 8-channel pipettor.



This figure presents the expected sizes of HLA amplicons. The purified HLA amplicons (lane 1-8) demonstrate clear, consistent bands, while the unpurified control (lane 9) shows more background, indicating the efficiency and consistency of the purification process using HLA PRO.

Figure 3. Amplicon concentration measured by Qubit after purification using the HLA Pro 8-channel pipettor.

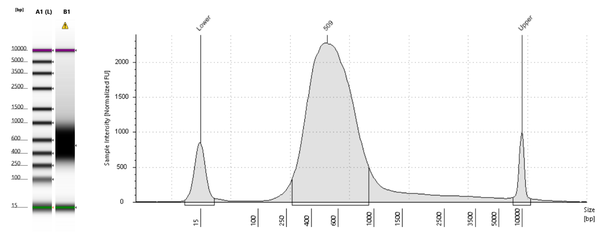


The chart represents eight replicates with concentrations ranging from 39.0 to 48.8 ng/µL. The dashed red line indicates a lower threshold for next step. Amplicon concentrations show consistency across replicates, demonstrating the reliability of the purification process performed by HLA PRO.

The library preparation script generated high-quality libraries, with optimal fragment sizes and ideal concentrations.

The HLA PRO system carried out the library preparation script, resulting in sequencer-ready fragments. The sequencing libraries exhibited good yield (with final concentration >0.5ng/uL) and contained fragments of appropriate sizes (average size 450 bp -950bp) for the Ion Torrent NGS system. The entire library preparation procedure was typically 6.5 hours, with hands-on time minimized to under 0.5 hours.

Figure 4. Representative TapeStation analysis of sequencing libraries prepared by the HLA PRO system.



The average fragment sizes range from 450 to 950 base pairs, as determined by multiple tests. These fragment sizes are optimal for loading onto the Ion Torrent sequencer, ensuring efficient sequencing and high-quality data. The sharp, consistent peaks in the TapeStation profile indicate successful library preparation and appropriate fragment distribution for NGS.

Sequencing results showed robust 11-loci coverage, a minimum read depth of 200, and high concordance rates above 99%, ensuring accurate typing across all sample sizes.

Table 1. Key Exon Coverage and Allele Concordance for 11 HLA Loci Analyzed by TypeStream Visual (TSV) Across Various Sample Sizes.

Sample Size	A	B	C	DPA1	DPB1	DQA1	DQB1	DRB1	DRB345	(%) Allele Concordance
8	528	465	487	891	263	577	671	414	354	**99.31%**
8	713	548	528	980	511	651	861	498	324	**100%**
13	476	589	583	400	192	623	513	450	337	**100%**
32	897	784	774	1005	310	633	561	519	469	**100%**
35	903	1102	945	420	271	719	546	861	767	**99.84%**
48	897	935	812	734	278	658	604	722	609	**99.76%**

The table summarizes sequencing coverage for an 11-loci HLA typing assay from libraries of different sample sizes. TypeStream software was used to analyze the results, with robust sequencing coverage observed across all loci. A minimum read depth of 200 was maintained across key exon regions, ensuring accurate and high-quality HLA typing.

Concordance is defined when at least one common allele matches between experimental and reference typings, while discordance occurs when no match is found at the 6-digit (Field-3) level. All sample sizes show high concordance rates, with values above 99%, demonstrating reliable typing accuracy across the dataset.

Conclusions

The HLA PRO system automates the generation of a multiplexed HLA library consisting of sequencer-ready fragments derived from amplified genomic DNA, specifically for use with the Ion Torrent Sequencer. By automating this process, the HLA PRO significantly reduces the hands-on time required for library preparation prior to sequencing on the Ion Torrent platform under 0.5 hr.

- The HLA PRO amplicon purification script efficiently purified PCR amplicons, resulting in high yields with minimal variation.
- The library preparation script generated high-quality libraries, with optimal fragment sizes and ideal concentrations.
- Sequencing results showed robust 11-loci coverage, a minimum read depth of 200, and high concordance rates above 99%, ensuring accurate typing across all sample sizes.

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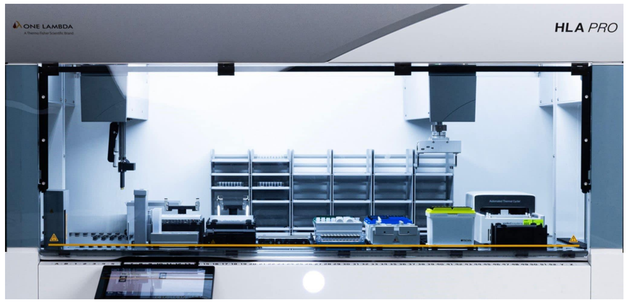

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