# Automation of LABType™ HLA Typing using the HLA PRO™ Liquid Handling Platform

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## Abstract

**Purpose:** Adapt the LABType SSO workflow on the HLA PRO platform to allow fully automated runs of up to 384 samples.

**Methods:** An automation script was developed and optimized for the LABType SSO workflow, covering all steps post-PCR denaturation to data acquisition by Luminex. We evaluated minimum and maximum sample configurations for accuracy and assessed reproducibility (16 samples) for representative kits on multiple instruments.

**Results:** Accuracy and reproducibility studies show 100% concordant typing.

## Background

Human leukocyte antigen (HLA) typing by reverse sequence-specific oligonucleotide (rSSO) hybridization is a rapid, economical method for genotyping transplant donors and recipients. Biotinylated PCR products hybridize to dye-coded bead arrays and are detected with streptavidin–phycoerythrin (SAPE) conjugates on Luminex FLEXMAP 3D® (FM3D) analyzers. The manual LABType™ workflow requires repeated hands-on steps between amplification and data acquisition. We transferred the complete post-PCR workflow to the HLA PRO platform, enabling unattended processing of up to four 96-well plates (384 reactions) per run.



**Figure 1.** The HLA PRO platform. Inset: automation deck with 8-channel pipetting arm and labware hotels.

### INTEGRATED SYSTEM COMPONENTS

**8-channel pipetting arm —** performs all reagent and sample transfers (rSSO beads, SAPE, denaturation–neutralization buffers on deck)

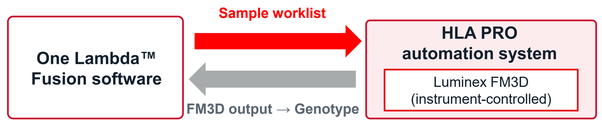
**Shakers and centrifuges —** on-deck bead pelleting and resuspension

**Thermal cycler (Applied Biosystems™) —** controlled incubation of assay intermediates

**Luminex FM3D reader —** side-deck loading and automated acquisition

**Robotic gripper arm —** plate transport between storage, deck, and reader

**Figure 2.** Software integration: One Lambda™ Fusion generates worklists and interprets FM3D output for genotype assignment.




## Materials and methods

**Samples.** Fully characterized in-house reference DNA representing HLA class I and class II alleles was amplified with LABType™ SSO and LABType™ CWD kits per kit-specific protocols; amplicon quality was verified by gel electrophoresis. Products were pooled and homogenized to a uniform source, aliquoted to minimize freeze–thaw variability, used within 30 days, and dispensed at 5 µL per well of 96-well assay plates.

**Study design.** Accuracy was assessed at maximum throughput (four 96-well plates, 384 reactions per kit) and minimum throughput (8 samples per product on one plate), each on two independent HLA PRO instruments. Reproducibility used 16 well-characterized samples replicated across well positions of three 96-well plates plus one water-control plate, tested on both instruments across multiple days in morning and overnight runs.

**Figure 3.** Assay workflow. All post-PCR steps run unattended on HLA PRO.




**Data analysis.** Hybridized plates were read on the LABScan3D™ / FLEXMAP 3D® platform. Data were analyzed with HLA Fusion™ v4.7 (xPONENT® v4.3; FluentControl™ v3.6 or higher). Close or inconclusive reactions were reviewed with cutoff adjustments applied per routine HLA Fusion analysis procedures.

### Acceptance criteria (all studies)

| Parameter | Criterion |
|---|---|
| HLA typing concordance | 100% vs. reference typings |
| Failed reactions | ≤ 5% of valid samples |
| Bead count per region | ≥ 75 (Classic SSO) · ≥ 50 (CWD) |
| Control TMFI | Positive / negative controls within product specification |
| Workflow completion | Start-to-finish without error |

## Results

Following optimization of the HLA Pro LABType workflow, the finalized Script v1.4 demonstrated strong and consistent performance for both accuracy and reproducibility testing.

•**Maximum-throughput accuracy:** 96-sample, four-plate testing met all predefined acceptance criteria. Confirmatory testing with high bead-density CWD kits demonstrated 100% HLA typing concordance, acceptable bead counts, and sample failure rates below the 5% threshold across both HLA Pro instruments.

**Table 1.** Maximum-throughput accuracy — 384 reactions per kit (96 samples × 4 plates), two instruments

| Kit | Instrument I | | | | Instrument II | | | |
|---|---|---|---|---|---|---|---|---|
| | Conc. | Beads | Failed, n (%) | $LB_{95}$ | Conc. | Beads | Failed, n (%) | $LB_{95}$ |
| SSO-B | 100% | 100 | 4 (1.0%) | 0.976 | 100% | 100 | 4 (1.0%) | 0.976 |
| SSO-DP | 100% | 100 | 0 (0%) | 0.992 | 100% | 100 | 0 (0%) | 0.992 |
| B-CWD / ACWD | 100% | 50 | 2 (0.5%) | 0.984* | 100% | 50 | 4 (1.0%) | 0.976 |

Conc. = HLA typing concordance (target 100%). Beads = reported beads/region. $LB_{95}$ = one-sided 95% lower confidence bound on the pass proportion (Clopper–Pearson).

**Figure 4.** Bead-count distribution across the four-plate ACWD (high bead density) run. Every well met the ≥50 beads/region criterion.




**Minimum-throughput accuracy:** Final 8-sample testing using four CWD products on both instruments achieved 100% concordance, acceptable bead counts, 0% sample failure, and successful completion of the automated workflow.

**Table 2.** Minimum-throughput accuracy — 8 samples per product, one plate,4 CWD kits, two instruments

| Kit | Instrument I | | | Instrument II | | |
|---|---|---|---|---|---|---|
| | Conc. | Beads | QC | Conc. | Beads | QC |
| RSSOW1A | 100% | 100 | ✓ | 100% | 100 | ✓ |
| RSSOW1B | 100% | 100 | ✓ | 100% | 100 | ✓ |
| RSSOW1C | 100% | 100 | ✓ | 100% | 100 | ✓ |
| RSSOW2B1 | 100% | 100 | ✓ | 100% | 100 | ✓ |
| General workflow — start-to-finish without error | | | | PASS — both instruments | | |

QC ✓ = failed-reaction criterion (≤5%) met; all runs completed without error.

**Reproducibility:** Following implementation of the final script modification, all subsequent morning and overnight runs across both instruments met acceptance criteria. Successful runs demonstrated 100% HLA typing concordance, acceptable bead counts, and sample failure rates within specification across multiple days and operating conditions.

**Table 3.** Reproducibility — three morning runs per instrument, two instruments (6 runs per kit)

| Kit | Concordance (target 100%) | Beads/region (≥50 / ≥75) | Failure rate (≤5%) |
|---|---|---|---|
| RSSO1B | 100% · 6/6 runs | 100 | Passed · 6/6 |
| RSSO2P | 100% · 6/6 runs | 100 | Passed · 6/6 |
| RSSOW1C | 100% · 6/6 runs | 50 | Passed · 6/6 |

Overnight runs across both instruments also met all acceptance criteria (data not shown)

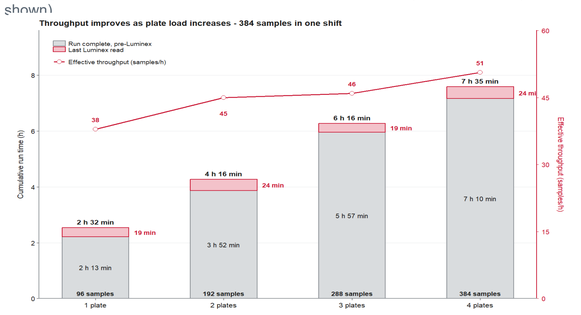



**Figure 5.** Walk-away run time and effective throughput by plate load.

Overall, the finalized HLA Pro LABType workflow was accurate, reproducible, and robust across instruments, sample loads, and testing conditions, supporting reliable automated processing of LABType SSO and CWD assays.

### CONCLUSIONS

- 100% concordance with reference HLA typings in every study, on both instruments, at minimum and maximum load.
- All QC criteria met, worst-case failed-reaction rate 1.0% vs. the ≤5% criterion; bead counts and control TMFI within specification.
- Reproducible across instruments, days, well positions, and morning/overnight shifts.
- 384 samples processed in 7 h 35 min of walk-away time

*Note: This product is intended for General Laboratory Use. It is the customer's responsibility to ensure that the performance of the product is suitable for the customer's specific use or application*

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