

Automation of barcoded NGS-Turbo® HLA-typing using INTEGRA MIRO CANVAS

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Introduction

NGS-Turbo® is a fast and reliable high-resolution HLA-typing technique that is used during time-sensitive procedures. Recently, NGS-Turbo® has been updated to include barcodes to exclude residual DNA from previous samples in accordance with ASHI guidelines. The INTEGRA MIRO CANVAS microfluidics system is an easy-to-use automation instrument (Figure 1). As it is crucial that HLA-typing is performed without error and with minimal hands-on time, a simplified, hands-off workflow for the automation of NGS-Turbo® on INTEGRA's MIRO CANVAS microfluidics system is demonstrated here (Figure 2).



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Figure 1. INTEGRA MIRO CANVAS automated microfluidics system.

Methods

A set of 24 DNA samples from the Genetic Testing Reference Material Coordination Program HLA58 panel was analyzed by NGS-Turbo®. For each sample, a single NGSgo-Turbo® Amp amplification was performed, which was used for both a manual and MIRO CANVAS automated NGS-Turbo® Prep library preparation. The prepared libraries were sequenced with Oxford Nanopore Technologies (ONT) MinION R10.4.1 flow cells on a GridION instrument. Data was processed using super accuracy basecalling and finally analyzed using NGSengine® 4.0.

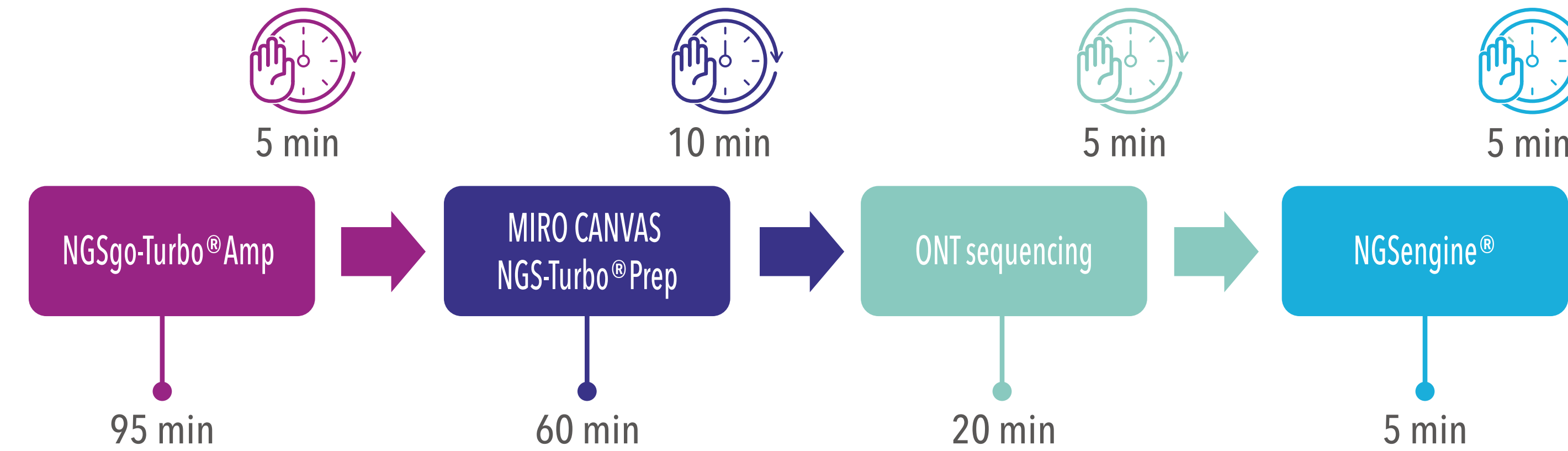


Figure 2. Simplified workflow for the automation of NGS-Turbo® on INTEGRA's MIRO CANVAS. Time requirements for individual steps are indicated and hands are used to indicate time requirements for hands-on steps. To prepare the amplified sample for MIRO CANVAS automation, the NGS-Turbo® Prep and ONT reagents are loaded onto a single-use MIRO CANVAS cartridge using easy-to-follow instrument prompts.

Results

The performance of the manual and automated NGS-Turbo® workflows was compared. Library yields were similar between the workflows and sequencing pore occupancy was slightly reduced in the automated vs the manual workflow (average 69% vs 77%) (Figure 3). All 3rd field typing results were 100% concordant. Furthermore, no significant differences were observed in the NGSengine® data statistics between the manual and automated workflows (Figure 3). Time to completion of the automated workflow is 3 hours, compared to 2.5 hours for the manual workflow. Even though the automated workflow is longer, the hands-on time is only 25 minutes, which is half that of the manual workflow.

Conclusion

Without losing library yield, sequencing output or data quality, automation of NGS-Turbo® including barcodes is successfully accomplished on the MIRO CANVAS. The automated workflow is more user-friendly, removes user-to-user variability and reduces hands-on time by 50%, freeing up precious time for laboratory staff during on-call procedures.

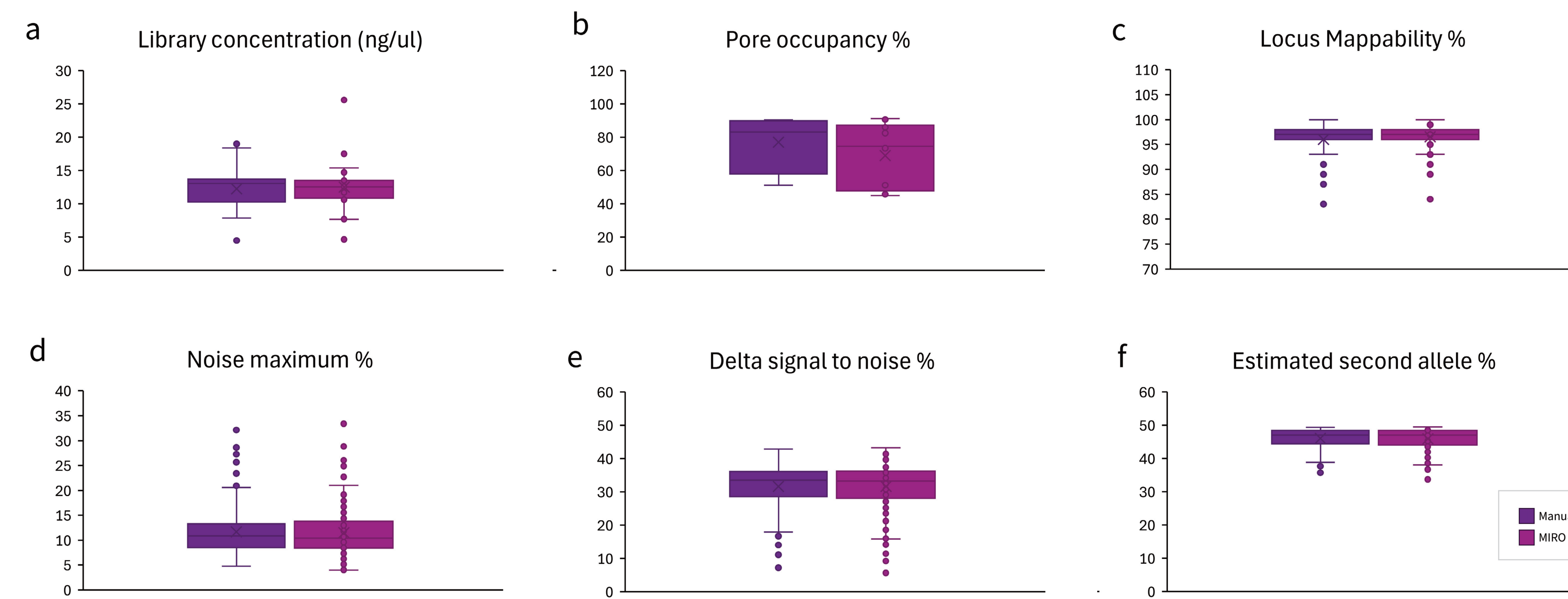


Figure 3. Comparative analysis between the manual and automated MIRO CANVAS NGS-Turbo® workflows. (a) Library yields in ng/ul, (b) Pore occupancy is an indication of the number of ONT sequencing pores versus all available pores during sequencing, NGSengine® data statistics; (c) Locus mappability %, (d) Noise maximum %, (e) Delta signal to noise % and (f) Estimated second allele %, are indicated.

