

NGS-Capto-HLA: Long-Fragment Hybrid Capture-Based HLA-Typing using Oxford Nanopore Technologies Sequencing

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Introduction

Amplification-based typing strategies are fast and reliable, but hybrid-capture-based strategies are more tolerant towards polymorphisms in primer/probe binding sites. We have developed a hybrid-capture-based HLA-typing prototype assay, named: NGS-Capto-HLA. Our assay contains a diverse panel of HLA binding probes that cover all classical HLA class I and II genes, as well as HLA-E, -F and -G (Figure 1). Moreover, NGS-Capto-HLA enriches long HLA fragments (between 3-5 kb) that, when combined with long-read sequencing, enable complete phasing. In this study, we aimed to further optimize the existing NGS-Capto-HLA workflow to increase sample throughput while reducing total processing time.

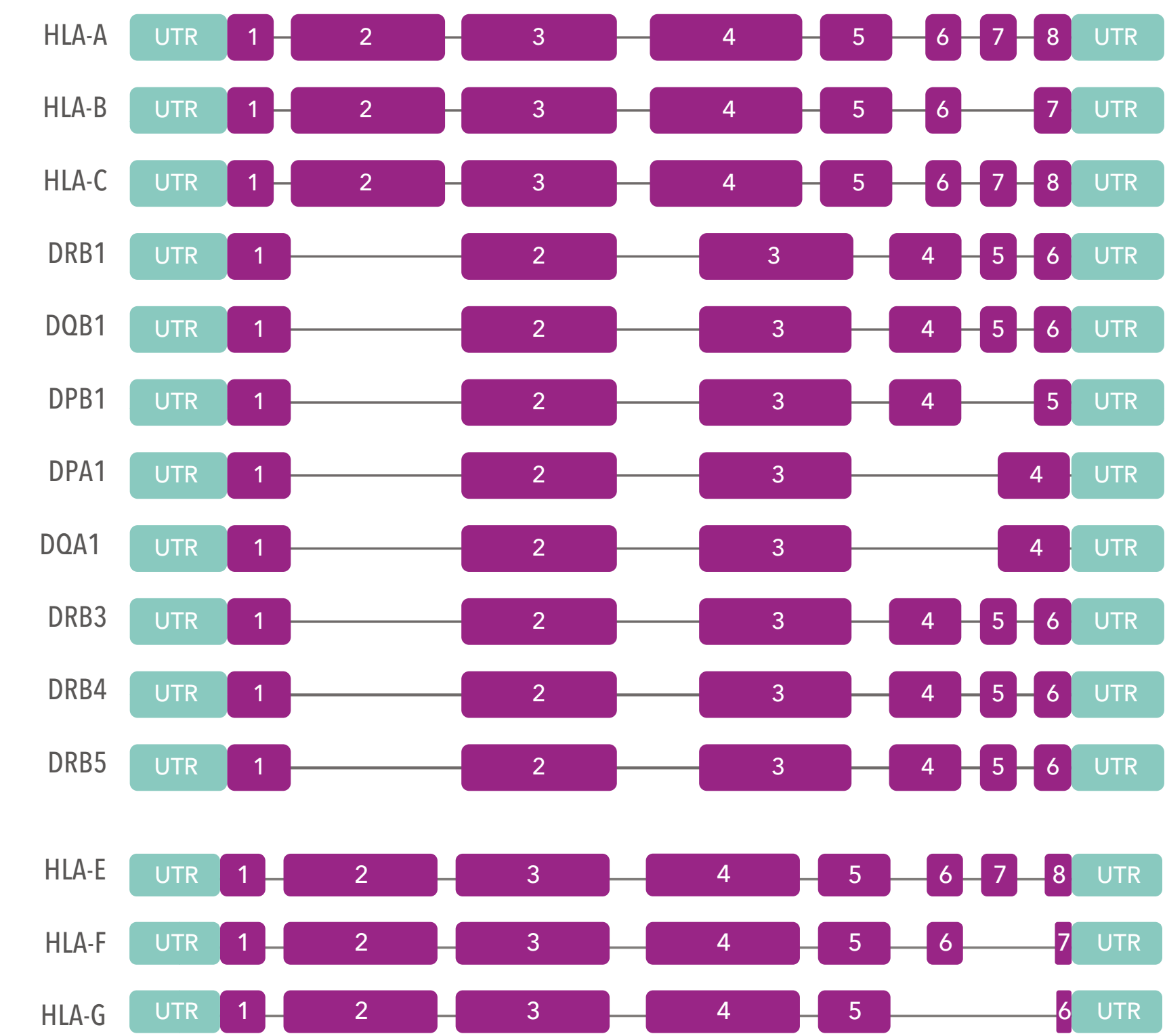


Figure 1. NGS-Capto-HLA achieves full coverage of all 11 classical HLA genes, as well as non-classical genes HLA-E, -F and -G.

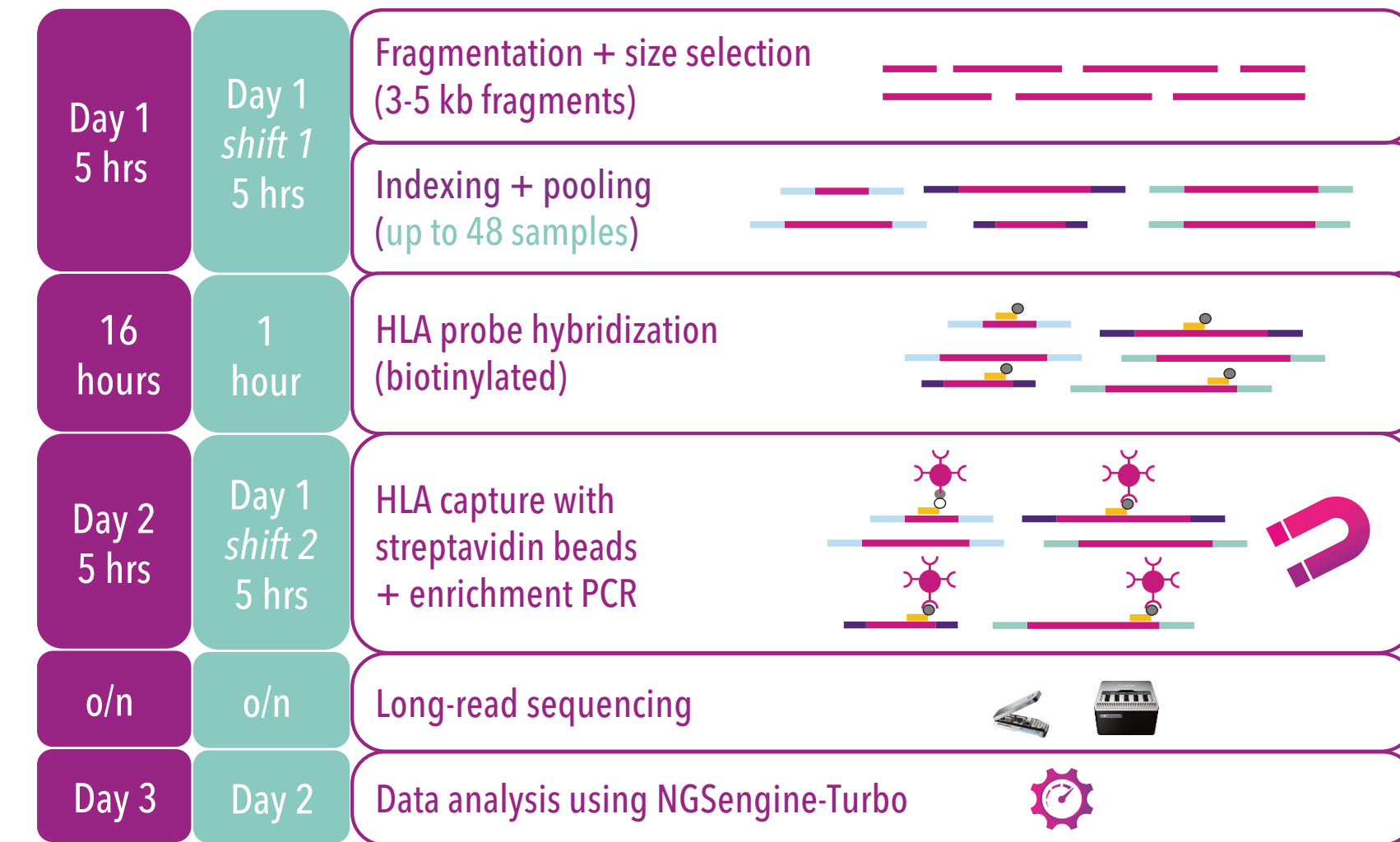


Figure 2. Schematic overview of the principle and turn-around times of different steps in the NGS-Capto-HLA workflow. Original and optimized timelines are indicated in purple and green respectively.

Methods

The original NGS-Capto-HLA workflow is described in Figure 2 - purple timeline. This two-day workflow features a 16-hour probe hybridization step and allows for pooling of 8 samples per hybridization reaction. The novel protocol evaluates performance after 1 hour of probe hybridization with up to 48 samples per hybridization reaction (Figure 2 - green timeline). Performance of the updated workflow was assessed in NGSengine-Turbo®, using typing concordance, on-target sample mappability, locus mappability, and noise level percentages as indicators of assay specificity.

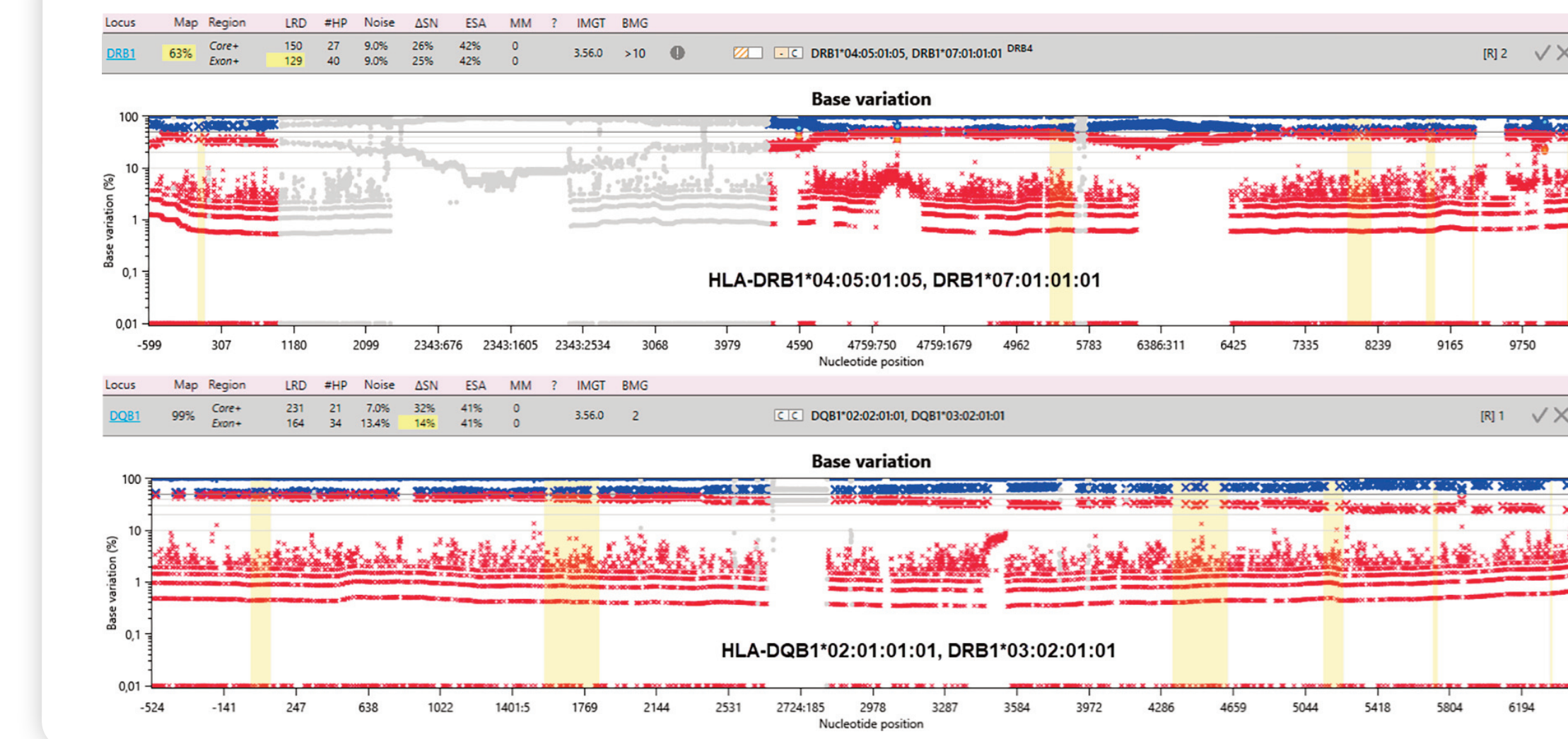


Figure 3. Two representative base variation plots showing sequencing results of difficult allele combinations of the HLA-DRB1 and HLA-DQB1 loci. Yellow and white areas in the base variation plot indicate exonic and intron regions, respectively. Grey regions are ignored from analysis.

Results

Performance metrics were highly comparable across all conditions. Two representative base variation plots demonstrate excellent data quality of difficult allele combinations of the HLA-DRB1 and HLA-DQB1 loci (Figure 3). Full typing concordance (100%) was achieved for the original and the conditions with 48 samples per reaction. Typing concordance dropped to 97% in the 8 samples, 1-hour hybridization condition (Figure 4D), however, as could be confirmed in later repetitions of the assay this was caused by a technical issue. Generally, combining a reduced hybridization time with an increased sample number yielded high-quality data and HLA-typing concordance, comparable to the original protocol (Figure 4).

Conclusion

The NGS-Capto-HLA prototype assay allows for flexible sample pooling and hybridization time. Pooling more samples per reaction will benefit high throughput laboratories. Additionally, laboratories working in shifts profit from shorter hybridization times that reduce the workflow duration to 2 days. These key enhancements will be critical for facilitating the implementation of NGS-Capto-HLA in clinical testing laboratories.

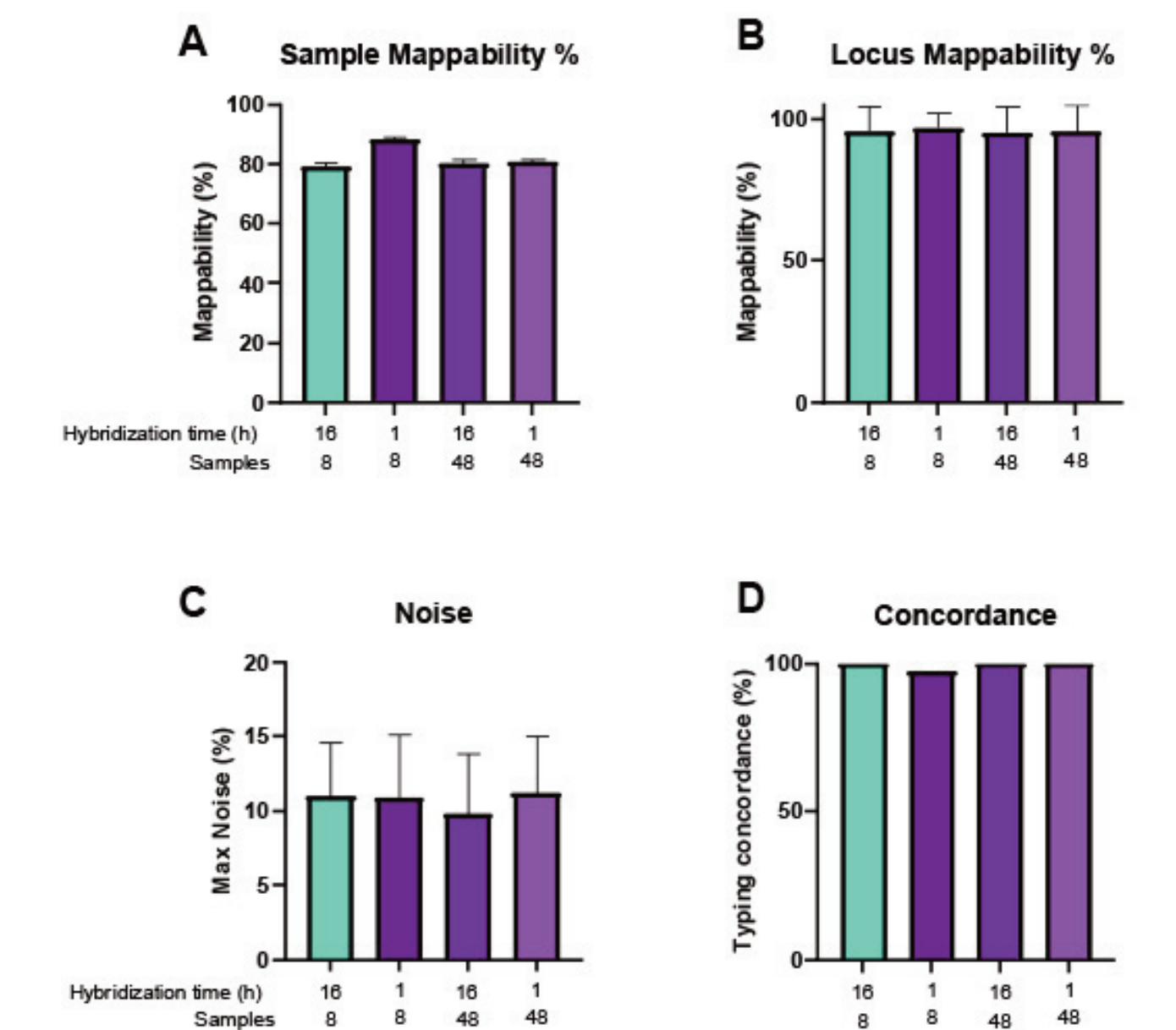


Figure 4. Performance of NGS-Capto-HLA with reduced hybridization times and increased samples, based on (A) on-target mappability of sequenced reads (B) percentage of mapped reads per identified locus (C) percentage of noise levels in the ONT sequence data and (D) typing concordance with available pre-type.

