

# Facilitating high-throughput HLA typing: Single-well, low-volume amplification with full exon coverage of 11 HLA loci

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## Introduction

To facilitate automated high-throughput, high-resolution HLA typing, we developed a single-well amplification prototype assay for 11 HLA genes (Figure 1). The amplification primers and master mix, which can be used in a volume as low as 4 µl before adding DNA, were designed to achieve a balanced amplification of all exons of the 11 relevant HLA loci (Figure 2).

## Methods

The single-well amplification assay, using 7 ng genomic DNA (gDNA) in 1,75 µl in 4 µl of amplification master mix, was used to amplify 58 diverse cell line samples from the Genetic Testing Reference Material Coordination Program (GeT-RM HLA58, Coriell Institute). Library preparation was performed using the NGSgo® Library Full Kit. Sequencing was performed on a MiniSeq (Illumina) platform and the data was analyzed using an experimental version of NGSengine®. Typing results were compared to available pre-typing information (4<sup>th</sup> field resolution), obtained using a combination of NGSgo®-ProntoAmp, NGSgo Library Full Kit, and sequencing on an Illumina MiniSeq platform.

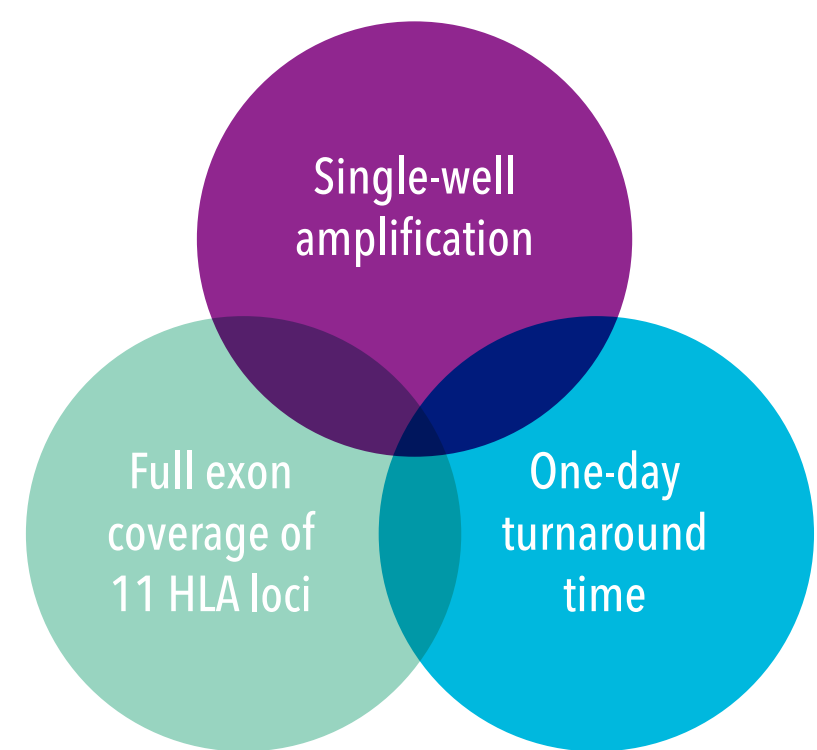


Figure 1. Amplification assay requirements.

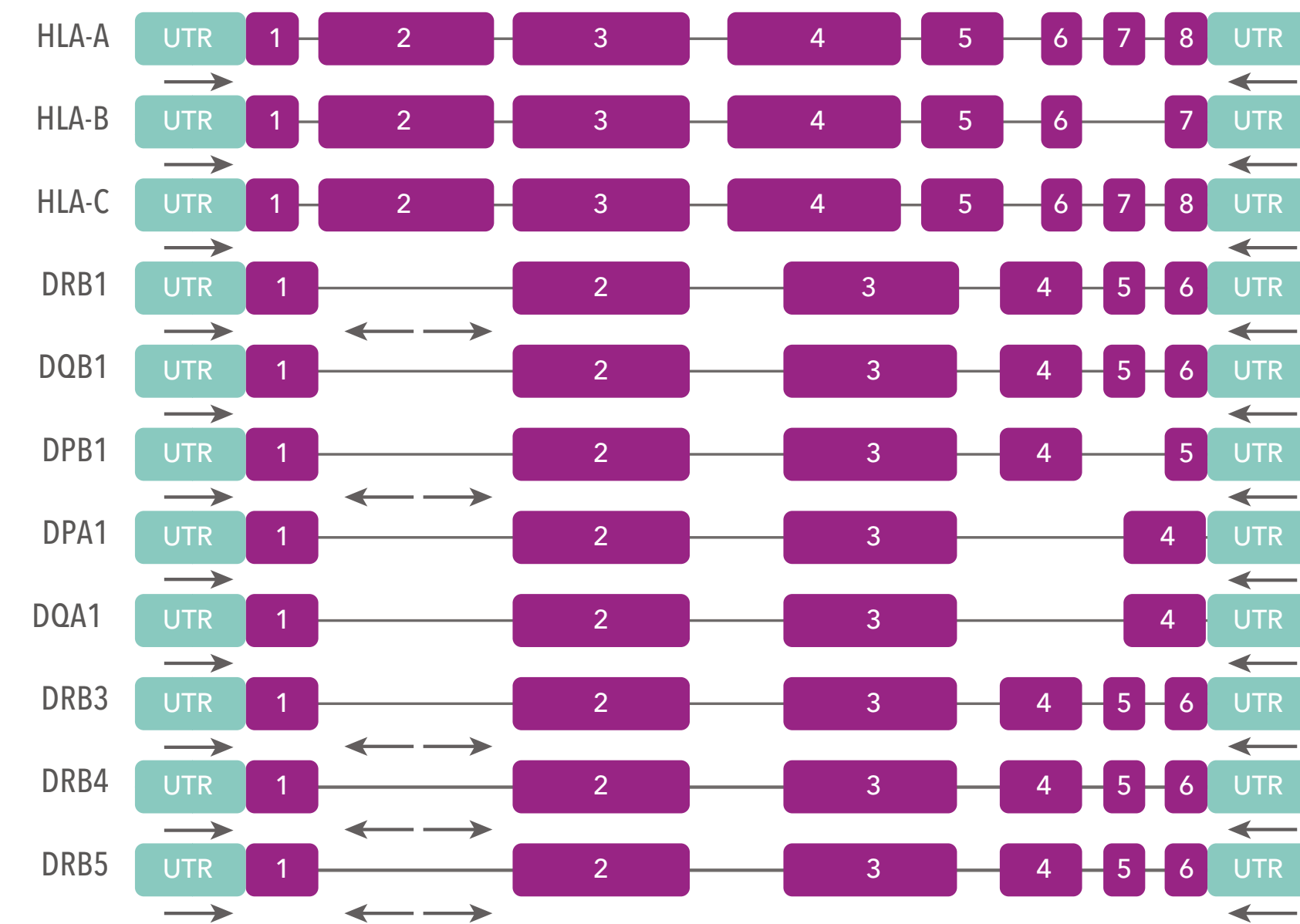


Figure 2. Amplification coverage of the assay, showing full exon coverage of 11 HLA loci. Purple boxes indicate amplified exons, arrows indicate amplification primers.

## Results

- Amplification of 11 HLA genes of 58 diverse samples obtained:
- 100% typing concordance (3<sup>rd</sup> field) for 11 HLA loci (Table 1)
  - 100% typing concordance (4<sup>th</sup> field) for 8 HLA loci (Table 1)
    - DRB1 and DPB1 showed lower concordance due to differences in the analyzed regions between the prototype assay and the pre-typing assay
    - DRB4 showed lower typing concordance due to extended coverage of the gene by the prototype assay
  - High-quality sequencing data with a clear discrimination between signal and noise
  - Balanced amplification of challenging loci such as DRB1\*04 heterozygotes and DQB1\*02/03 alleles combined with DQB1\*05/06 alleles (Figure 3)
  - gDNA to FASTQ in under 24 hours
    - Amplification: 2 hours 15 minutes
    - Library preparation: 3 hours 30 minutes
    - Sequencing: 18 hours

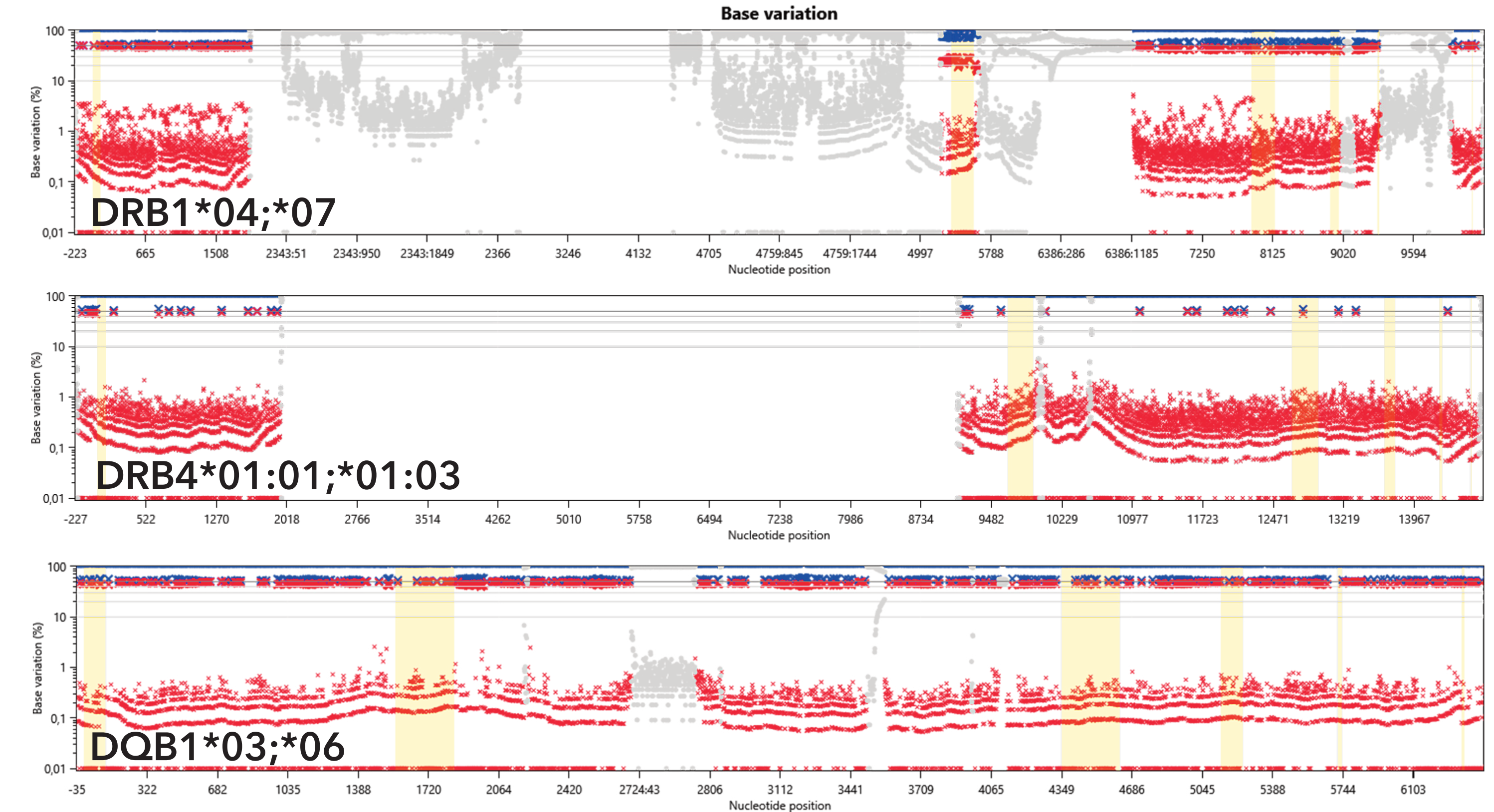


Figure 3. Y-axis shows base variation (%) on a logarithmic scale. Large crosses represent heterozygous positions, small crosses indicate noise at homozygous positions. Grey positions marked are ignored from analysis.

	HLA-A	HLA-B	HLA-C	DRB1	DRB3	DRB4
Number of samples	58	58	58	58	36	31
3 <sup>rd</sup> field concordance	100%	100%	100%	100%	100%	100%
4 <sup>th</sup> field concordance	100%	100%	100%	52%*	100%	97%

	DRB5	DQB1	DPB1	DQA1	DPA1
Number of loci	14	58	58	58	58
3 <sup>rd</sup> field concordance	100%	100%	100%	100%	100%
4 <sup>th</sup> field concordance	100%	100%	72%*	100%	100%

Table 1. Number of samples analyzed and concordance at 3<sup>rd</sup> and 4<sup>th</sup> field resolution.  
\* The analyzed regions between this assay and the pre-typing assay are different for these loci, causing discrepancies in the 4<sup>th</sup> field typing results.

## Conclusion

The prototype assay allows for balanced single-well amplification of all exons of 11 HLA loci, allowing for high-resolution HLA typing on Illumina platforms. Due to its simple single-well design, this assay is scalable for high-throughput and/or automation purposes. Additionally, alternative to Illumina sequencing, this assay is also compatible with ONT sequencing, further enhancing flexibility in sequencing (data not shown).