Rapid, robust and reliable high resolution HLA typing of 11 HLA loci using ont minion sequencing

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Aim

- Rapid HLA typing of deceased donors is crucial to ensure minimal graft deterioration and rejection post-transplant
- Here, NGS-Turbo®, a method for rapid HLA-typing in combination with Oxford Nanopore (ONT) MinION sequencing HLA typing concordance is evaluated

Methods

- Amplification of 11 HLA loci in 90 minutes, NGS-TurboAmp
- Custom library preparation was developed (Figure 1)
  - Long amplicons left intact
  - Barcoding possible for up to 96 samples
  - Duration of 2 hours, but can be performed in 30 minutes for single samples
- GeT-RM HLA58 HLA reference panel (Coriell Institute)
- Sequencing for 24h on R10.4 MinION flow cells, 24 samples per flow cell
- Basecalling with high accuracy algorithm performed on the fly
- Data analysis with prototype version of NGSengine

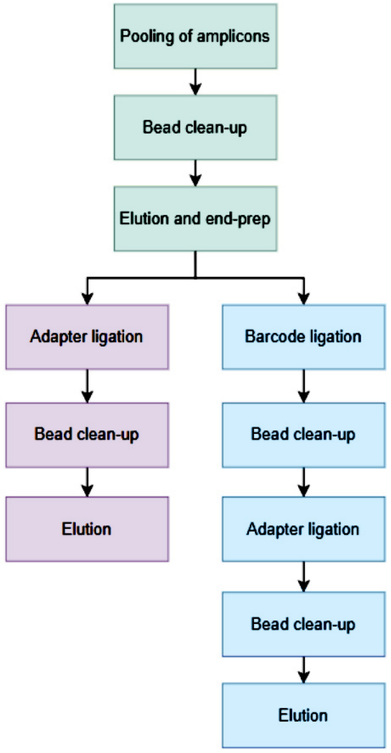

Figure 1: Schematic overview of library preparation procedure. In green the shared part between single sample and barcoded approaches. In purple and blue respectively are steps specific for the single sample and barcoded procedures.

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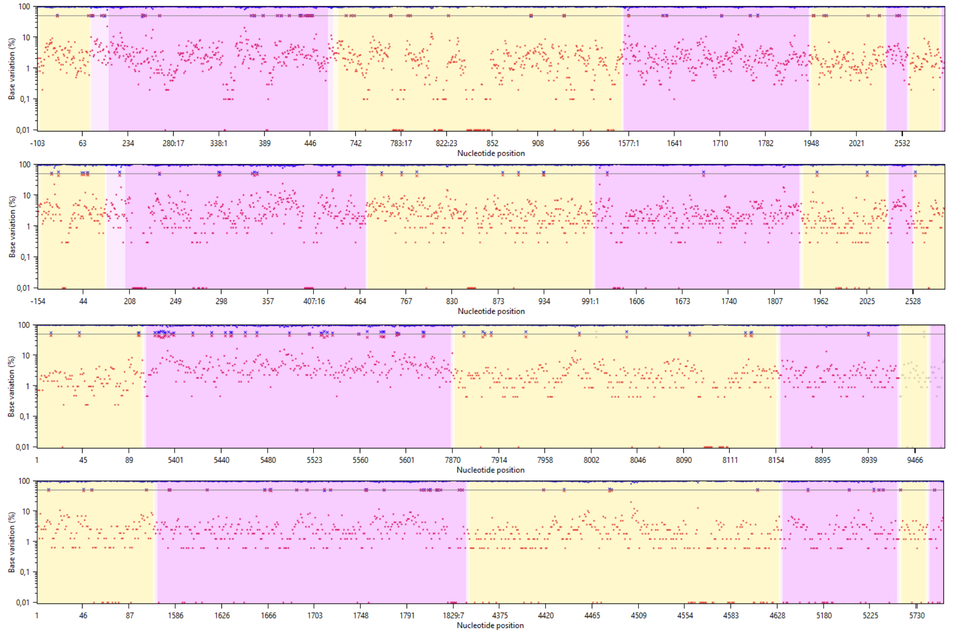
Results

- Sequencing resulted in an average N50 of 5.59kb for three runs, indicating long amplicons are indeed kept intact
- Two-field typing concordance of 96.3% was observed with 525/545 loci giving a correct typing result (Table 1, Figure 2)
- Typing issues were traced back to potential for optimization of the assay and software at two-, three- and four-field typing resolution
- Issues caused by sequencing artifacts were limited
- After optimization of assay and software performance, 19 erroneous results were rescued, increasing typing concordance to 99.8% (544/545 loci)

Table 1.
Concordance of HLA typing at different resolutions

	Typing resolution		
	Four fields	Three fields	Two fields
Samples tested (n)	58	58	58
Loci tested (n)	545	545	545
Correct typing results (n)	515	522	526
Concordance (%)	94.50	95.78	96.51
Erroneous typing results (n)	30	23	19
Assay/software related issues	27	22	18
Sequencing related issues	3	1	1
Concordance after optimization (%)	-	-	99.80

Figure 2:
Examples of exon+ base variation plots of concordant results. From top to bottom results are shown for HLA-A, HLA-B, HLA-DRB1, and HLA-DQB1.



Results

- A homopolymer in HLA-DRB3 exon 2 (Figure 3) caused a wrong typing

Conclusion

- The assays developed here allow for robust second field resolution HLA typing and can be applied in settings where time is a critical factor
- Two-field typing concordance of R10.4 ONT chemistry was 99.8% after optimization and is expected to improve with further developments to reagents, software and the introduction of R10.4.1 chemistry

Figure 3:
HLA-DRB3 base variation plot of discordant result with detailed sequence view