HLA TYPING USING NANOPORE TECHNOLOGY: A BETA TESTING STUDY

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Abstract

Aim: We explored the benefits of nanopore sequencing for HLA typing in a beta testing study in collaboration with Omixon Ltd. and Oxford Nanopore Technologies (ONT) Ltd.

Methods: Ninety-nine genomic DNA samples extracted from peripheral blood and buccal samples, previously typed at a high resolution level using a 2 generation NGS method, were tested using the Omixon NanoTYPE™ multiplex kit. Protocols with enzyme-based and magnetic bead purification were also compared.

Results: Results for class I loci (HLA-A, -B, -C) were 99% concordant at three-field typing. One new allele was detected and one HLA-B locus dropout (HLA-B*51:01:02) was observed. For two samples, typing of HLA-B*44:03:01 was reported as an ambiguous combination with HLAB*44:336. Results for HLA-DRB1/3/4/5 and HLA-DQB1 were also 99% concordant with previous results. We observed two HLA-DQB1*03:01:01 dropouts, one HLA-DQB1*03:19 dropout, and one HLA-DQB1*04:02 dropout. We observed one instance of an incorrect typing of HLADRB1*04:58N, resulting from low amplification of HLA-DRB1*04:01:01. Results for HLA-DQA1, -DPA1, and -DPB1 loci were 100% concordant with previous results. The library preparation time was significantly reduced using enzyme-based purification in place of magnetic beads. We also typed three samples in a mock deceased donor typing scenario, with adjustments to sequencing times, to test the accuracy and reproducibility of the results. NanoTYPE™ was able to deliver three-field, high-resolution typing with 100% concordance for these samples.

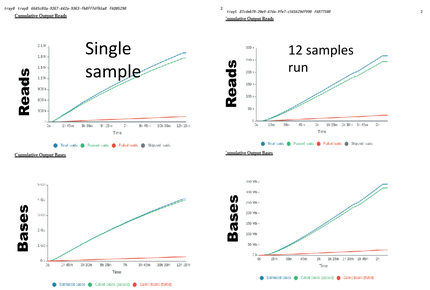
Conclusion: Nanopore-based HLA typing using the NanoTYPE™ kit is advantageous in allowing shorter turnaround times for multiple samples

and is more cost-effective than traditional NGS methods. With refinements to the method to prevent allele dropouts, it will constitute a useful tool for HLA high-resolution typing. Reduction of the PCR amplification time would make this a feasible typing method to provide high-resolution HLA typing of deceased donors, resulting in greater availability of organs for highly sensitized patients.

Nanotype

12 samples	1 Sample	
30 minutes	30 minutes	DNA Extraction
2hours	2 hours	HLA Amplification 11 loci
• 5min • 10 min • 10 min	• min • 3min • 1min	• Amplicon Purification • Quantitation • Normalization
• 3 min • *Pooling and Purification* 20min	• 3min • 5 min	Library Preparation • Fragmentation • Barcoding • Rapid Adapter attachment Preparation of Sequencing reaction
• 2min	• 2min	Flow cell check
• 5min • 5min	• 5min • 5min	Priming and Loading Sequencing
12 hours	2 hours	
~ **16** hours	~ **5** hours	

Cumulative reads & Percentage of Pass/Fail reads



Discrepancies

Allele drop-outs	B*51:01:02 (1); DQB1*03:01:01 (2); DQB1*03:19 (1); DQB1*04:02:01 (1)
Missed calls	Call: DRB1*04:58N; actual: DRB1*04:01 Call: DQA1*05:01, 05:23; actual: DQA1*05:01 Call: B*44:03:01 or 44:336; actual: B*44:03
Other limitations	Aberrant splice sites intron DRB4*01:03:01N Exon 5 for all DRB1/3/5

Overall Assay Performance

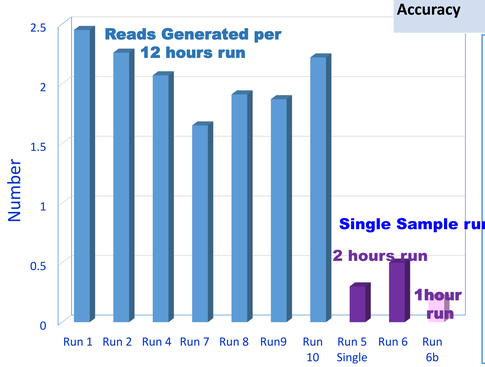
Metric	HLA-A	HLA-B	HLA-C
Accuracy	100%	99%	100%
	-DRB1	DRB345	-DQA1
Accuracy	99%	99%	99.8%
	-DPA1	-DPB1	-DQB1
Accuracy	100%	100%	98%

Project Summary

	Reads Generated	Passed Bases	Estimated Bases	Failed Bases	Read Length average (kbp)	Median Qscore	Total time
Run 1	2.45 M	5.25 Gb	5.29 Gb	296.27 Mb	2.69	12	12h 4 m
Run 2	2.26 M	4.78 Gb	4.98 Gb	314.98 Mb	2.69	>12	12h 2 m
Run 4	2.07 M	4.53 Gb	4.74 Gb	263.02 Mb	2.94	12	12h 2m
Run7	1.65 M	3.89 Gb	3.83 Gb	324.98 Mb	3.0	12	12h 6m
Run 8	1.91 M	3.98 Gb	4.08 Gb	277.99 Mb	3.2	12	12h 2m
Run 9	1.87 M	3.62 Gb	3.81 Gb	181.16 Mb	2.94	>12	12h 2m
Run 10	2.22 M	4.91 Gb	4.66 Gb	362.22 Mb	3.0	12	12h 6m
Single samples	Reads Generated	Passed Bases	Estimated Bases	Failed Bases	Read Length average (kbp)	Median Qscore	Total time
Run5	268 k	321.19 Mb	339.25 Mb	25.33 Mb	3.0	10	**2h 4m**
Run6	463.52k	612.84 Mb	612.84 Mb				**2h 3m**
Run6b	163.37 k	176.39 Mb	183.36 Mb	10.22 Mb	3.0	10	**1h 2m**

Expected Reads: 110-140 Mb which is 50000 to 60000 per sample
Q-score : Range between **Q7** and **Q15**

12 Samples per Sample run



Conclusions

- ❑ Nanopore-based HLA typing using the NanoTYPE™ kit is advantageous in allowing shorter turnaround times for multiple samples and is more cost-effective than traditional NGS methods
- ❑ Q score and amount of reads obtained ***Pass*** the limited range.
- ❑ With refinements to the method to prevent allele dropouts, it will constitute a useful tool for HLA high-resolution typing
- ❑ . Reduction of the PCR amplification time would make this a feasible typing method to provide ***high-resolution HLA typing*** of ***deceased donors***, resulting in greater availability of organs for highly sensitized patients