Improvements of Omixon NanoTYPE HLA Typing Using Oxford Nanopore Technologies: Addressing the DQ3 Drop-Out

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Introduction

In 2023, our lab received approval for the validation of Omixon NanoTYPE HLA typing using Oxford Nanopore Technology (ONT) from ASHI and the New York State Department of Health. During our validation process from 2022 to 2023, we identified a significant drawback: DQ3 dropouts. This issue was particularly concerning for DQ5/DQ3 or DQ2/DQ3 combinations, with dropout rates for the DQB1*03 allele being 30.8% and 6.7%, respectively (see the following table). We began testing patient samples in early 2024 and have been closely monitoring this issue.

Results

Results

Out of the 390 samples, 191 contained at least one DQ3 allele. Among these, 52 samples had a DQ5/DQ3 combination, and 59 samples had a DQ2/DQ3 combination. Notably, no DQ3 dropout was observed in either of these two groups.

DQB1 Combination	# of Dropped DQ3	Total #	Drop out rate
DQ5/DQ3	0	52	0%
DQ2/DQ3	0	59	0%

DQB Combination	# of Dropped DQ3	Total #	Drop out rate %	Comments
		10	00.0	A
DQ5/DQ3	4	13	30.8	Amplification Imbalance
DQ2/DQ3	1	15	6.7	Amplification Imbalance

Is this continually going to be an issue for us?

Materials and method

DQB1 combination	# of Dropped DQ3	Total #	Drop out rate %
DQ5/DQ3	0	52	0
DQ2/DQ3	0	59	0

DQ3 Homozygous	0	24	0%
DQ3 heterozygous	2	191	1.05%

Conclusion

Since our initial validation of the NanoTYPE method two years ago, significant improvements in both the amplification kit and software have been made by Omixon, effectively resolving the DQ3 dropout issue and enhancing overall performance. The observed DQ dropouts in two runs are not due to a failure of the kit.

In the Run on 6/19/2024 ** Out of 11 samples, three missed DQ locus completely. • One DQ4 dropout with DQ4/DQ6 combination. • One DQ3 dropout with *DQ3/DQ6* combination **In another run on 7/17/2024:**

Out of 11 samples, one DQ3 dropout observed with *DQ6/DQ3* combination.

Our lab routinely uses

blood for clinical testing

the Maxwell RSC (Promega) to extract DNA the VeritiPro Thermal cycler (ThermoFisher) for DNA amplification.

The Omixon NanoTYPE assay involves

Interpretend with the second secon v2 kit)

rapid barcoding library preparation A nanopore sequencing (MinION flow cells), Addata-analysis using NanoTYPER software.

To date, we have tested 390 patient samples.

Why did we choose MinION?

Runs	Missed one DQ	Missed all DQ	Total samples	Failing rate of DQ
<mark>6/19/2024</mark>	2	3	11	45.5%
<mark>7/17/2024</mark>	1	0	11	9.1%
39	3	3	390	1.6%

** We believe the DQ failed in this particular run due to factors other than amplification kit.

Overall

✤ 24 DQ3 homozygous samples were identified, with 18 confirmed through SSP high-resolution

Reference

ASHI Quarterly 2024 Q1 p37-41 **TECHNOLOGISTS' AFFAIRS COMMITTEE** Validation of Omixon NanoTYPE HLA typing using Oxford Nanopore **Technologies (ONT)**

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MinION has a small footprint and portable

(see the right picture)



No capital cost for instrument

Sample numbers for each run is flexible (1-24)

tests. After June, we stopped for confirmation.

Only two DQ3 dropouts were observed out of 191 samples which contained at least one DQ3.

(*Refer to the table in the top right corner*)



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