Pilot Study of the KIR Complete MX Reagent with Oxford Nanopore Sequencing

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Methods and Materials

KIR Complete MX from GeneDx is designed to amplify all exons and introns of KIR loci from genomic DNA using a multiplex amplification strategy. 16 of the 32 DNA samples were extracted using Agilent Salt precipitation method. The assay amplifies 2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 2DP1, 3DL1, 3DL2, 3DL3, 3DS1, and 3DP1 in three multiplex PCR reactions per sample. The amplicons were used for library preparation, followed by sequencing on the ONT platform. Data was analyzed using NGSengine software.

Aim

The aim of this study was to evaluate the performance and feasibility of the KIR Complete MX reagent in generating high-quality Killer-cell Immunoglobulin-like Receptor (KIR) genotyping data using Oxford Nanopore Technologies (ONT) sequencing. This pilot study assesses sequencing accuracy and overall workflow efficiency to determine the reagent's suitability for potential clinical applications.

Conclusions

This pilot study demonstrates the feasibility of using the KIR Complete MX reagent for KIR genotyping on the ONT platform. The KIR NGS assay is designed to amplify full-length KIR genes, including exons and introns, with target regions of up to approximately 17 kb. Our results indicate that DNA integrity and sample quality are critical factors for successful amplification and accurate KIR genotyping.

Results

A total of 32 pre-typed samples were analyzed, and the results are shown in the tables. Of the 32 samples, 16 were previously extracted DNA samples. Concordant KIR genotyping results were obtained for 8 of 16 previous extracted DNA samples (50%). One sample failed during testing. The remaining 7 of 16 samples showed partial discordance, with discrepancies observed in the typing of specific KIR genes when compared to consensus results.(Table 1) We think the discordant results in several samples were likely due to not optimal DNA quality. KIR NGS assays are designed to amplify full -length KIR genes, including exons and introns (up to 17kb).

In a subsequent experiment, KIR genotyping was performed on 16 newly collected peripheral blood samples. All samples were collected within 2–3 days prior to testing, and DNA was manually extracted using the Agilent DNA Extraction Kit. All 16 samples (100%) demonstrated concordant KIR genotyping results compared with the consensus data. (Table 2)

Sample ID	Extraction Method	Test method	2DL1	2DL2	2DL3	2DL4	2DL5	2DS1	2DS2	2DS3	2DS4	2DS5	3DL1	3DL2	3DL3	3DS1	2DP1	3DP1
227		Consensus	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	NEG	POS	POS	POS	POS	POS	POS
227	NA	GenDX NGS	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	NEG	POS	POS	POS	POS	POS	POS
249		Consensus	NEG	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	NEG	NEG	POS
249	NA	GenDX NGS	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	NEG	NEG	POS
251		Consensus	POS	POS	NEG	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS
251	NA	GenDX NGS	POS	POS	NEG	POS	POS	NA	NEG	POS	NEG	NA	NEG	NEG	POS	NEG	POS	POS
252		Consensus	POS	POS	POS	POS	POS	NEG	POS	NEG	POS	POS	POS	POS	POS	NEG	POS	POS
252	NA	GenDX NGS	POS	POS	NEG	POS	POS	NA	NEG	NEG	POS	POS	POS	POS	POS	NEG	POS	POS
255		Consensus	NEG	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	NEG	POS
255	NA	GenDX NGS	NEG	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	NEG	POS	POS
256		Consensus	POS	NEG	POS	POS	POS	POS	NEG	NEG	POS	POS	POS	POS	POS	POS	POS	POS
256	NA	GenDX NGS	POS	NEG	POS	POS	POS	POS	NEG	NEG	POS	POS	POS	POS	POS	POS	POS	POS
257		Consensus	POS	NEG	POS	POS	POS	NEG	NEG	NEG	POS	POS	POS	POS	POS	NEG	POS	POS
257	NA	GenDX NGS	POS	NEG	POS	POS	POS	NEG	NEG	NEG	POS	POS	POS	POS	POS	NEG	POS	POS
258		Consensus	POS	NEG	POS	POS	NEG	NEG	POS	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
258	NA	GenDX NGS	POS	NEG	POS	POS	NEG	NA	POS	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
259		Consensus	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS
259	NA	GenDX NGS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS
260		Consensus	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	NEG	POS	POS	POS	POS	POS
260	NA	GenDX NGS	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	POS
266		Consensus	POS	NEG	POS	POS	POS	POS	NEG	POS	NEG	POS	NEG	POS	POS	POS	POS	POS
266	NA	GenDX NGS	POS	NEG	POS	POS	POS	POS	NEG	POS	NEG	POS	NEG	POS	POS	POS	POS	POS
268		Consensus	POS	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	NEG	POS	POS
268	NA	GenDX NGS	POS	POS	POS	POS	POS	POS	POS	NA	POS	POS	POS	POS	POS	NEG	POS	POS
269		Consensus	POS	NEG	POS	POS	POS	POS	POS	NEG	NEG	POS	NEG	POS	POS	POS	POS	POS
269	NA	GenDX NGS	POS	NEG	NEG	POS	POS	NA	NEG	NEG	NEG	NA	NEG	NEG	POS	NEG	POS	POS
270		Consensus	POS	POS	NEG	POS	NEG	NEG	POS	NEG	POS	NEG	POS	POS	POS	NEG	NEG	POS
270	NA	GenDX NGS	POS	POS	POS	POS	NEG	POS	POS	NA	POS	NA	POS	POS	POS	NEG	NEG	POS
271		Consensus	NEG	POS	NEG	POS	NEG	NEG	POS	NEG	POS	NEG	POS	POS	POS	NEG	NEG	POS
271	NA	GenDX NGS	NEG	POS	NEG	POS	NEG	NA	POS	NEG	POS	NEG	POS	NEG	POS	NEG	NEG	POS
272		Consensus	POS	NEG	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS
272	NA	GenDX NGS	POS	NEG	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS

Table 1

Sample ID	Extraction Method	Test method	2DL1	2DL2	2DL3	2DL4	2DL5	2DS1	2DS2	2DS3	2DS4	2DS5	3DL1	3DL2	3DL3	3DS1	2DP1	3DP1
21152		Consensus	NEG	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	NEG	POS
21152	AGILENT	GenDX NGS	NEG	POS	NEG	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	NEG	NEG	POS
72639		Consensus	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72639	AGILENT	GenDX NGS	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72651		Consensus	POS	POS	POS	POS	NEG	NEG	POS	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72651	AGILENT	GenDX NGS	POS	POS	POS	POS	NEG	NEG	POS	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72652		Consensus	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72652	AGILENT	GenDX NGS	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72653		Consensus	POS	NEG	POS	POS	POS	POS	NEG	NEG	NEG	POS	NEG	POS	POS	POS	POS	POS
72653	AGILENT	GenDX NGS	POS	NEG	POS	POS	POS	POS	NEG	NEG	NEG	POS	NEG	POS	POS	POS	POS	POS
72657		Consensus	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	NEG	POS	POS	POS	POS	POS
72657	AGILENT	GenDX NGS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	NEG	POS	POS	POS	POS	POS
72659		Consensus	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72659	AGILENT	GenDX NGS	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72660		Consensus	POS	POS	POS	POS	POS	NEG	POS	POS	POS	NEG	POS	POS	POS	NEG	POS	POS
72660	AGILENT	GenDX NGS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	NEG	POS	POS	POS	NEG	POS	POS
72669		Consensus	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72669	AGILENT	GenDX NGS	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72671		Consensus	POS	NEG	POS	POS	POS	POS	NEG	NEG	POS	POS	POS	POS	POS	POS	POS	POS
72671	AGILENT	GenDX NGS	POS	NEG	POS	POS	POS	POS	NEG	NEG	POS	POS	POS	POS	POS	POS	POS	POS
72702		Consensus	POS	POS	POS	POS	NEG	NEG	POS	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72702	AGILENT	GenDX NGS	POS	POS	POS	POS	NEG	NEG	POS	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72703		Consensus	POS	POS	POS	POS	POS	NEG	POS	POS	POS	NEG	POS	POS	POS	NEG	POS	POS
72703	AGILENT	GenDX NGS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	NEG	POS	POS	POS	NEG	POS	POS
72708		Consensus	POS	NEG	POS	POS	POS	POS	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72708	AGILENT	GenDX NGS	POS	NEG	POS	POS	POS	POS	NEG	NEG	POS	POS	POS	POS	POS	POS	POS	POS
72712		Consensus	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72712	AGILENT	GenDX NGS	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72713		Consensus	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72713	AGILENT	GenDX NGS	POS	NEG	POS	POS	NEG	NEG	NEG	NEG	POS	NEG	POS	POS	POS	NEG	POS	POS
72718		Consensus	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS
72718	AGILENT	GenDX NGS	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS	POS	POS	POS	POS	POS	POS

Table 2

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