KIR Complete MX: Multiplexed whole gene genotyping of all KIR genes using ONT sequencing

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Introduction

The Killer Immunoglobulin-like Receptor (KIR) genes in the Leukocyte Receptor Complex (LRC) region of chromosome 19 are studied due to their regulatory function in Natural Killer cells and their interactions with HLA class I. The length of the genes, intergenic homology, variable copy numbers and haplotypes make elucidating allelic KIR variety challenging. The KIR Complete MX prototype assay is a full gene multiplexed amplification strategy for KIR genes (Figure 1). High-to-allelic typing resolution is enabled by Oxford Nanopore Technologies (ONT) and PacBio long-read sequencing.

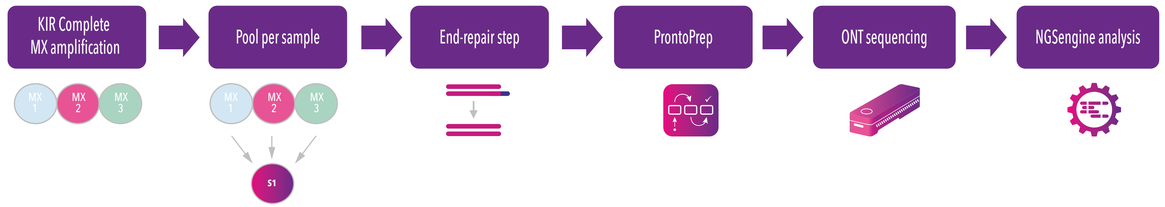

Figure 1. KIR Complete MX workflow

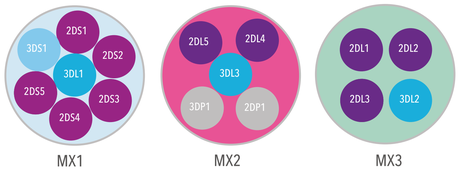

Figure 2. KIR complete MX multiplexing

Locus	2DL1	2DL2	2DL3	2DL4	2DL5	2DP1	2DS1	2DS2	2DS3	2DS4	2DS5	3DL1	3DL2	3DL3	3DS1	3DP1
Concordant/Expected	6/6	4/4	6/6	7/7	6/6	6/6	4/4	4/4	4/4	7/7	4/4	6/6	7/7	7/7	4/4	7/7
Mappability	93	91	89	95	87	91	94	94,5	86,0	79,5	86,5	85,5	89,0	90,0	89,0	96,0
Exon lowest read depth	976	848	454	841	625	1799	592	336	421	261	178	218	609	1221	226	323
Exon delta signal to noise	39,1	N/A	32,5	32,2	42,1	38,0	N/A	N/A	40,8	37,9	35,1	36,3	38,2	38,0	N/A	29,8
Exon estimated second allele	48,6	N/A	48,0	46,0	46,3	44,7	N/A	N/A	46,6	45,0	42,6	48,1	47,4	48,4	N/A	41,7

Table 1. Achieved typing concordance and metrics within the seven sample test panel.

Methods

An optimized three-mix multiplex strategy (Figure 2) was evaluated on a panel of seven human cell line gDNA (Coriell Institute) samples selected for high KIR gene content and allelic variation. Pre-types were available based on whole gene Illumina data. Per mix 60 ng of DNA was used in a 28 cycle PCR followed by amplicon pooling, end repair, and cleanup steps. Then per sample 10 µl was used in the GenDx NGS-ProntoPrep workflow. Libraries were sequenced on ONT GridION using R10.4.1 flow cells and SUP accuracy basecalling. Data was analyzed with GenDx NGSengine and the KIR-IPD 2.13 reference library.

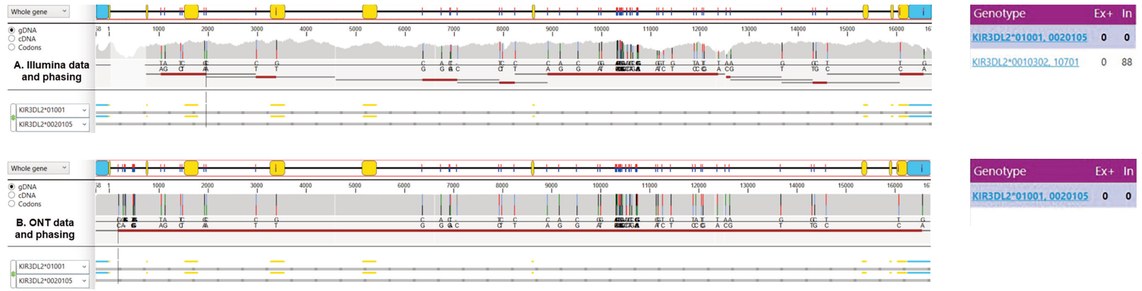

Figure 3. Long read data enables genotyping ambiguities to be resolved. In Illumina data (a), phasing (red horizontal line) was interrupted, leading to a first field ambiguity. In ONT data (b) the phasing is uninterrupted, leaving just one possible genotype.

Results

For all samples tested, all expected KIR genes were reported in the analysis. All KIR alleles included yielded concordant genotypes up to five digits (89/89) and acceptable quality metrics (Table 1). Correct calling of these alleles was dependent on sufficient reads generated, where 15.000 reads per sample is recommended. Several exon mismatches were reported, all of which corresponded with the pre-type data. It was noted that genotype ambiguities, a relatively common occurrence in Illumina-based KIR typing, were fully absent due to the long-read phasing offered by the ONT sequencing data (Figure 3).

Conclusion

The KIR Complete MX prototype assay is capable of accurately genotyping all KIR genes and alleles in the tested high variation sample set without genotype ambiguities. Larger panels of (externally) pre-typed samples with different sources will be used to further verify the assay's robust performance.

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