Whole Gene KIR genotyping on a commercially available human HLA variation reference panel

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Introduction

KIR genes are regulating NK cell response. While KIR is often studied at a haplotype level (figure 1) more evidence in relation to the allelic content has become available in the recent past. In KIR-IPD database 2.11, 1535 alleles were included, adding over 400 alleles since 2019. When implementing allelic level typing in a new laboratory, a source of pre-typed, high quality genomic (cell-line) DNA allows for easy reference to the correct implementation of the method. In this study we aim to provide complementary KIR typing data to the earlier published (Bettinotti et al. 2018) high-resolution HLA data of the HLA58 polymorphism panel from the Coriell institute.

Material & Methods

NGSgo-AmpX KIR (figure 2a.) (GenDx) was used for the whole gene amplification of 8 KIR genes (KIR2DL1, 2DL2, 2DL3, 2DL4, 3DL1, 3DS1, 3DL2, and 3DL3). Amplicons were confirmed on agarose gel and then equimolarly pooled based on Qubit Br (Thermo-Fisher) quantification of representative positive samples. The NGSgo Library Full Kit (GenDx) was used to process pools for sequencing on MiSeq (figure 2b.) (Illumina) using a 2x151 cycle kit. Resulting data was analyzed using NGSengine version 2.21 (GenDx) employing IPD-KIR 2.9.0. Samples containing genotype ambiguities or novel alleles were confirmed by PacBio library preparation workflow and sequencing on PacBio Sequel II (figure 2c.) (Pacific Biosystems). Again, resulting data was processed using NGSengine.

Results

Out of 58 samples, 57 samples (98%) amplified robustly, with a single sample (NA17286) showing weak or no amplicons, likely due to low DNA quality. Out of 464 sample-locus combinations amplified, 82% showed a positive PCR product and 18% were negative on agarose gel, reflecting the variability in KIR gene content. Out of the positive products, 98% showed high quality Illumina sequencing data whereby 79% resulted in an unambiguous allele assignment. Another 19% showed genotype ambiguities despite full gene coverage, mostly due to the phasing limitations of short read sequencing (figure 3, 4). Finally, 8 putative new alleles were found.

Upon sequencing genotype ambiguities and putative new alleles on PacBio Sequel II (figure 5), all putative new alleles were confirmed, 88% of ambiguities were confirmed to be the 'best match' as called MiSeq data analysis, 8 were in fact the ambiguous result from the MiSeq analysis and 1 locus (NA10005, KIR3DL1) showed a novel allele with an exonic mismatch not detected by MiSeq due to lack of phasing.

Conclusion

Full gene sequence data was achieved for all but 1 of the samples included. Sequencing using both Illumina and PacBio systems thereby resulted in the full characterization of a 57 sample panel for 8 KIR genes. The genotypes found will be made available through the GenDx website.

Figure 1. A general outline of the KIR genes present in the most frequent haplotypes, including an indication which genes are included in this study

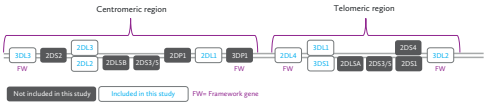


Figure 2. NGSgo AmpX KIR kit and both of the sequencers used in these studies

Figure 3. Results of all loci included in amplification after Illumina sequencing, negative and positive unambiguous loci were accepted, positive ambiguous and new allele loci were included in sequencing on PacBio.

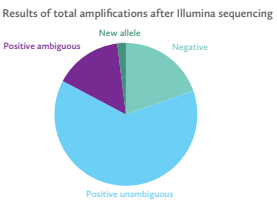


Figure 4. Visualization of the numbers of different alleles found (y-axis) per KIR gene (x-axis) within the 57 samples

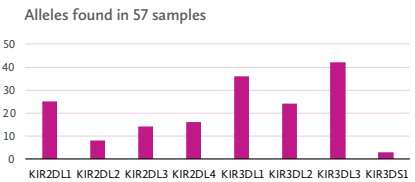
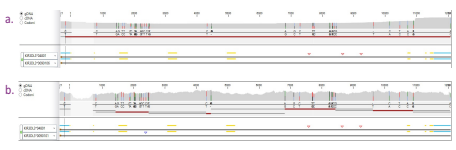


Figure 5. The resulting data of PacBio (a) and Illumina (b) sequencing visualized by the NGSengine software for the same sample and locus (NA17285, KIR3DL3). The red bar, representing the phasing, illustrates the benefit of the addition of PacBio data in those cases where Illumina sequencing cannot resolve genotype ambiguities.



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