The killer immunoglobulin-like receptors (KIR) genes belong to the immunoglobulin superfamily and are widely studied due to the critical role they play in coordinating the innate immune response to infection and disease. KIR gene clusters are found on chromosome 19q13.4, in a span of ~150 kb region. The content of these clusters varies in number and type of gene alleles based on linkage disequilibrium and determines each haplotype as A (inhibitory) or B (activating) 1). Thus resolving individual genotypes of members of the KIR gene clusters, within and between the haplotypes of a given individual is important for biological interpretations of its function, phenotype, and disease.

Current genome-wide analysis methods or PCR based approaches for genotyping KIR genes in population studies, have been limited in their ability to acquire phased, extended, and complete genomic sequences that are long enough to assemble haplotypes with high confidence. Highly accurate, contiguous, long reads, like those generated by SMRT Sequencing, when combined with target-enrichment protocols, provide a straightforward strategy for generating complete de novo assembled KIR haplotypes. We have explored two different methods to capture the KIR region; one applying SMRT Sequencing, the other using NimbleGen SeqCap enrichment.

**Complex Organization of KIR Haplotypes**

Figure 1. Polymorphic Gene Content of KIR Haplotypes: Gene Deletion, Insertions, and Hybridizations (ONVs)

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Figure 2. Recombining approach for target enrichment of fosmids to capture haplotypes of interest from a human genome in a tiling approach.

Figure 3. Workflow for SMRT® Sequencing of a full-length fosmid library preparation from a pool of fosmids belonging to a single haplotype and both haplotypes enriched from targeted regions of interest from genomic DNA and an automated DNA analysis pipeline.

Figure 4. Reconstructing based method for isolating a tiling of targeted fosmids (~35 kb – 50 kb) was combined with SMRT Sequencing. 16 haplotypes assembled from 8 individuals (available in NCBI). Partial reference, available for homologous sample 5701-9005 was 100% concordant with PacBio assembly.

Figure 5. Barcoding options for targeted sequencing

Figure 6. A. Known Haplotypes
B. Coverage showing alignment of captured reads >8.5 kb against the known reference haplotypes
C. Coverage of >8.5 kb reads against known haplotypes when data is aligned against all known full-length KIR sequences
D. De novo assembled phased sequence, red indicates assembly error that results in a contig of mixed phase

Conclusions

- SMRT Sequencing generated highly accurate long reads necessary for simultaneous genotyping and haplotyping of complex KIR regions
- SMRT Sequencing of target-enriched fosmids provided >35-50 kb contiguous sequences for improving/establishing reference database with imputation-free information
- NimbleGen SeqCap, a scalable alternative, validated known KIR haplotypes of a known sample in a reference guided assembly of 5-8 kb enriched DNA