**KIR Genotyping using a Real Time Assay and FLX 454**

Abha Chopra¹, Don Cooper¹, Laghima Sharma¹, Anne Plauzolles², Mark. W. Watson¹, Andri Rauch⁴, Silvana Gaudieri¹,², Simon Mallal¹,³

¹Institute for Immunology and Infectious Diseases, Murdoch University, Murdoch Western Australia; ²University of Western Australia; ³Royal Perth Hospital, Perth, Western Australia, ⁴University Hospital Bern, Switzerland

**Background:**
- Killer immunoglobulin-like receptor (KIRs) are cell surface receptors expressed on natural killer (NK) cells.
- KIRs regulate the function of NK cells by interacting with HLA Class I ligands.
- KIR and HLA interactions are associated with malignant, infectious and autoimmune diseases.
- KIR can be grouped into two haplotypes – A and B.

**Introduction:**
- KIR are a highly polymorphic family of 16 genes on chromosome 19q13.4.
- KIR genes are identified as both inhibitory and activating.
- KIR proteins are characterized as 2D and 3D (with two or three extracellular immunoglobulin-like domains) with long(L) and short (S) cytoplasmic tails.

**Method:**
- Previously published primers sets were evaluated for genotyping the KIR loci using the real-time assay using SYBR green. 15 primer sets were selected for validation.
- 24 samples from the UCLA KIR panel were selected for assay validation and subsequently 86 samples from the Swiss HIV Cohort study were genotyped.
- The process was automated on a 384 well plate which allowed four KIR genes for up to 94 samples to be tested on a single plate.
- A positive internal control GALC was included.
- Melt curve analysis was performed after real-time PCR.
- For FLX Sequencing, RNA was extracted from previously genotyped cells lines. New primers were designed to amplify the KIR genes: 2D L1-5, S1-5, P1 and 3D L1-3, S1, P1. Amplified products were sequenced on the long read 454 FLX platform.

**Results:**
- 100% concordance was achieved for all 15 KIR genes on 24 samples.
- Each KIR amplicon had a characteristic melting temperature (Tm) ranging between 79-85°C.
- GALC had Tm=75°C allowing easy discrimination between KIR and positive control peaks.
- Melting peaks were analysed to determine the presence/absence of KIR genes.

**Coverage obtained from FLX Sequencing:**

(example image of coverage plots)

**Conclusion 1:**
Real-Time KIR Genotyping using SYBR Green is-
- A simple and time-efficient technique that requires minimal post-PCR processing.
- Fast with analysis based on Ct values and melting temperatures.
- Able to easily determine presence/absence of KIR genes.
- A valid method to determine KIR haplotypes.

**Conclusion 2:**
The primers designed to amplify KIR coding sequences from RNA samples have captured most of the KIR genes. Further analysis is required to call alleles and understand why some genes were not captured and potential differences in expression between subjects.

**Acknowledgements**
We would like to thank all staff at IIID and Dr. Campbell Witt at RPH for his support.